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FOLLICULINIDS ON OYSTER SHELLS

By

E. A. ANDREWS
Johns Hopkins University
Baltimore, Maryland

INTRODUCTION

Drifting oyster larvae often survive by fastening their shells to those of preceding generations, making shell aggregates. Many other permanently sedentary organisms also attach themselves to oyster shells; thus arise oyster shell communities, in which we may find bacteria, diatoms, red, green and brown algae, protozoans, sponges, hydroids, actinians, bryozoa, pelecypods and ascidians as fixed permanent dwellers.

Moreover, these sedentary communities are frequented and made use of in various ways by animals of less sedentary habit, such as annelids, nematodes and platyhelminthes, rotifers, mollusca, acarida, crustacea, and some echinoderms and vertebrates.

Back in 1873 A. E. Verrill (22) named 190 kinds of animals as occurring on oyster beds, and Nelson (19) says: "The shell of an oyster is a veritable zoologist's paradise, for on it may be found representatives of every phylum of the animal kingdom."

That the ciliated protozoans, folliculinids, are wide-spread members of this oyster-shell community is to be demonstrated in the present paper.

WHAT ARE FOLLICULINIDS AND WHERE FOUND?

The family Folliculinidae has been separated from the Stentors as more complex in feeding apparatus and as dwelling long in secreted

tests having forms and sizes characteristic of different species and genera. Most of them have been found in the sea.

First seen on the coast of Denmark on algae in 1781, they have been found since on algae, flowering plants, stones, shells, wood, hydroids, bryozoa, serpula tubes and even on the surfaces of certain living crustacea. Various species have been recorded from the Arctic and the Antarctic, the Atlantic and the Pacific, the Indian Ocean, the White Sea, the Baltic, the Mediterranean and the Chesapeake Bay. As yet they are not on record from most of Asia, as well as most all Australia and all the coasts of South America. And there is but one record from Africa and the Pacific coasts of North America (7). Their world-wide distribution may be due in part to their habit of attaching themselves to solid objects, some of which float and may be carried by currents, and in part to their habit of escaping from their tests to swim free when they may be borne by currents. However, their occurrence in fresh waters in Switzerland, France, England and Uruguay needs some different explanation. May the adults survive on the feet of aquatic birds or in the hair of water-frequenting mammals? At present we have no knowledge of spores of folliculinids that might be carried by winds.

ADVANTAGES OF USING OYSTER SHELLS TO EXTEND KNOWN FACTS AS TO GEOGRAPHICAL DISTRIBUTION

Since folliculinids are taken in brackish as well as in more saline waters and since they are attached to various sorts of mollusc shells, it was to be expected that they would be found on oyster shells; and there is one mention of their being found in Europe on oyster shells together with other shells. That they may abound on oyster shells was put on record in 1880 by Ryder (20) when stationed with the U. S. Fish Commission steamer, *Fish Hawk*, at St. Jerome, St. Mary's County, Maryland. Forty-two and forty-three years later we found stones and oyster shells in parts of that same harbor thick set with two kinds of folliculinids.

As oysters are readily recognized and marketed, it seemed that an examination of oyster shells from various localities should be useful in extending the known geographical distribution of folliculinids in American waters, where they are little known.

Many zoologists in the United States and Canada were so kind as to send me random collections of oyster shells from the most northerly known beds in the Gulf of St. Lawrence, Prince Edward Island and Nova Scotia, down along the Atlantic shores of Maine, Massachusetts, New York, New Jersey, Maryland, Virginia, North Carolina, Florida,

Louisiana and Texas, as well as from the Pacific shores of California; in all these localities folliculinids were found on oyster shells.

TAXONOMY

In Europe folliculinids have been described as different species and genera, and here in America several of these different sorts have been recognized. Following the classification of Dons (12), rather than that of Kahl (16), but with consideration of the views of Fremiet (13), and of Hadzi (15), we here "lump" many diversities to reduce the forms in North America to the following ten: of the Eufolliculininae, we recognize here a form that seems like Mueller's (18) *Folliculina ampulla*, another that Hadzi (15) has described as *Metafolliculina andrewsi*, as well as the two new commensals, *Pseudoparafolliculina portitor* (6) and *Brachyfolliculina paguri* (3), and at Woods Hole Martin Burkenroad found what is probably the *Metafolliculina longicollis* of Hadzi (15); of the Semifolliculininae, we recognize *Lagotia viridis* (9), *L. simplex* Dons (12), *Parafolliculina violacea* (Giard) (4), *Parafolliculina amphora* Dons (2). In addition, Dons (10) in 1915 stated that his *Lagotia* (*semifolliculina*) *spirorbis* occurred on the eastern coast of North America.

Of these, *Folliculina ampulla* Mueller has not been reported previously from America; the peculiar tests found on oyster shells are near to Dons' figure and interpretations of than little-known and often erroneously identified species, but more study of the living animals is much needed. The second form, *Metafolliculina andrewsi*, has local differences in size and grouping of tests that may warrant separation from the form recognized by Hadzi. The third is an undescribed commensal of Limulus to be named *Pseudoparafolliculina portitor*. The fourth is a commensal of hermit crabs from Maine called *Brachyfolliculina paguri*, Andrews and Reinhard (3). The fifth, *Lagotia viridis*, includes the numerous synonyms recognized by Dons and may yet be subdivided. The sixth, *Lagotia simplex*, was found at Woods Hole, Massachusetts by Fremiet and more recently (7) on hermit crabs from California. The seventh, *Parafolliculina violacea*, everywhere agrees well with European forms as well as those mentioned from Woods Hole and from Beaufort by the present writer, but there is a marked variety with stalk in Long Island Sound. The eighth, *Parafolliculina amphora*, is everywhere in agreement with those described (2) from near Baltimore, Md. The ninth, *Lagotia spirorbis* has not yet been found again in America. The tenth, *Metafolliculina longicollis* Hadzi, though like the form from the French coast called *Folliculinopsis producta* Wright 1859 by Fremiet needs more study and may prove to be a variety of *M. andrewsi*.

GENERAL CONSIDERATIONS

While oyster shells serve to reveal the existence of these protozoans all along the Atlantic coasts of North America, there may be some doubt as to whether all the habitats were original, natural, or perhaps only recently acquired by aid of man. That is, oysters are carried from place to place to be planted and transplanted: from Connecticut to Long Island, and from more southern waters to more northern. However, it is not known if folliculinids can remain alive in these transports of oysters. Additions to the native fauna may also occur when folliculinids attach themselves to boats that pass from one body of water to another.

Previously folliculinids had been recorded only from the regions of Newport, R. I., Woods Hole, Mass., branches of the Chesapeake in Maryland, and Beaufort, North Carolina, but the examination of oyster shells has served to greatly extend the known distribution. In fact, we find that of the forty-five actual places in which folliculinids have been found on this continent, twenty-two are occurrences only on oyster shells, five on oyster shells and other objects, and of the remaining eighteen, some dozen, that is two-thirds, were merely subdivisions of the general occurrence on submerged flowering plants along the shores of side branches of the Chesapeake, near Baltimore, Md. Considering these as one locality, we find that of thirty-three different localities twenty-seven were places where the folliculinids occurred on oyster shells.

The number of shells taken at random from each of the 27 localities ran from 2–75, and of them a very variable percentage bore folliculinids; in three or four instances this was 100 percent; it was 50 percent or greater in seven instances; and it was lower in the rest. The entire number of shells examined was 600, and of these 200 bore folliculinids, that is, 33 per cent.

Some shells, in most all localities tested, bore folliculinids, but the following were samples taken that bore no folliculinids: a dozen shells from the upper part of the harbor at Woods Hole, Mass., June, 1942; shells bought as from the Patuxent R., Md., Jan. 1942; 18 shells off Sherwood Forest, West Round Bay, Md., July 28, 1943; shells from off Sandy Point, near Annapolis, Md., autumn, 1941; five shells from the Eliot River near Charlottetown, Prince Edward Is., Canada, Sept. 14, 1942; and eight shells from Frederick Cove, Conway Narrows oyster area, P. E. Is., Sept. 14, 1942.

Obviously, we cannot infer that folliculinids are absent from these localities until much more extensive samples are studied, for the distribution of folliculinids over an oyster area is probably similar to

that over the flowering aquatics along the shores of the Severn River branch of the Chesapeake Bay, where some patches of weeds were thickly populated and adjacent areas devoid of folliculinids.

The number of folliculinids found on one oyster shell ranged from one to several hundred.

It may be emphasized that inasmuch as part of the oyster shell is in contact with the bottom or with other shells and may be partly overgrown with organisms to which the folliculinids do not attach, the remaining areas of shells capable of bearing folliculinids may be small. Over such favorable areas the folliculinids often are scattered at random, but often they are aggregated in depressions or in protected regions of the shell surface, or within an empty shell.

The individuals may stand isolated, far apart, or may be grouped as in flocks with various densities from $\frac{1}{8}$ individual up to 6 individuals per sq. mm. Some groups have individuals in contact with, or even attached to, one another, but the amount of crowding depends upon the species. Some are more solitary and others more gregarious, their habits in settling being part of their specific nature.

Thus *M. andrewsi* is most prone to present some clusters attached by common "coletoderm," or cement, and *P. amphora* most apt to appear as isolated individuals. Dons (11) gives a photograph of small clusters of his *Parafolliculina roestensis* which he supposes due to one original settler giving rise to offspring that settled down close to the ancestor; however, in *M. andrewsi* we have observed large aggregates made by the nearly simultaneous settling of many young that were influenced by one another in clumping together. Probably the secretions, issuing from each as it swims and settles, influence mechanically others that are drawn to the same region.

While most all the recorded occurrences of folliculinids were made in the summer season (the vacation of a naturalist) some of those on oysters are the winter exceptions, and this seems of import in such side branches of the Chesapeake as the Severn River where, in the months of July and August, vast swarms of folliculinids may be found on submerged vegetation along the shores, coming after the annual growth of the plants has started and disappearing mysteriously before it ceases. The question of where these species exist in the winter is partly answered by the finding of both of them on oyster shells in the nearby beds of Round Bay, Md.

Maps published by the U. S. Coast and Geodetic Survey (21) indicate four small oyster bars in Round Bay that may total an area of about one square kilometer, or roughly 1,196,551 square yards; and the estimates made by Caswell Grave (14) in 1912 define a bed as

profitably workable for tongers at depths of 22 feet if the oysters on the bottom are about an average of 15 to the square yard. Since some tongers use those beds, we infer they may find at least 15 to the yard, or there may be about 17,948,265 usable oysters over the bottom of Round Bay (about eighteen million).

From our few observations it would seem that folliculinids may occur on about 30 percent of these oysters, or approximately on 6 million oysters. Again the number of folliculinids to be expected on an average oyster here is very uncertain, but it might be about thirty; so we suppose that about 180 million folliculinids are resident on the oyster shells of the Round Bay bars.

When in midsummer the water is very warm and the plants in the shallow waters along the shore are well grown, the great numbers of folliculinids found on their leaves may have migrated from the oyster beds; if Round Bay beds do not supply sufficient numbers there are also some miles of beds down the river from Round Bay to Annapolis, estimated in 1912 as 25 bars on both sides of the river in water 6 to 18 feet in depth, 998 acres in all, of which 100 acres might be made to produce oysters.

The hypothesis that folliculinids migrate from winter quarters on oyster shells to midsummer sojourn on submerged plants along shore is in harmony with the following observations: while in the eastern part of Round Bay there were many folliculinids on the oysters in April, there were but few in June, and in the western part, though abundant in December and in March, none were found in July 1943, these observations being based on a pocket-lens examination of some hundreds of shells and a thorough examination of eighteen shells, secured by the Maryland Department of Tidewater Fisheries; yet at the same time some were seen on leaves of potamogeton along the nearest shore, as well as up Weems Creek.

In the summer season much migration of folliculinids from lower to upper parts of plants was inferred, yet there were some instances of increase in numbers and possibly some seasonal outbursts of multiplication which might be bound up with conjugation, about which nothing is yet known in the Folliculinidae.

The same hypothesis of migration, with or without multiplication, may be applied to other arms of the Chesapeake where folliculinids, in the absence of rocky shores, are found in warm weather attached to elodea and other aquatic flowering plants near the shores. Thus the folliculinids found far up Middle River might owe their origin to immigrants from such oyster beds as those off Tolchester Beach, which, standing at the limit of oyster beds toward the fresh waters

of the head of the Bay, are subject to destruction in freshets, yet supplied in 1910 many thousands of bushels of marketable oysters at the rate of 91 bushels to the acre.

It is to be emphasized that while folliculinids were very abundant up Middle River, at Clark's Point in 1941, none could be found in 1942 at an earlier period of the summer; apparently, while conditions were favorable, the folliculinids had not yet arrived. While present in late November 1941, with water temperature 9° C and pH 7.8, they were not present late May to late July 1942, with water at 20–27° C and pH 8.7–8.3. One year they had been brought there by favorable winds and currents, but next year they had not yet been able to migrate there? Folliculinids are known to feed on bacteria, as well as on diatoms and some minute algae, but the conditions that favor a flourishing community are not known sufficiently to make successful attempts to culture these protozoans in the laboratory.

From an enumeration of the localities in which each of ten known species in North America are found, it appears that five of them may occur on oyster shells, one as a hypercommensal on the egg capsules of turbellarian worms attached to the gill plates of the arachnoid Limulus, two on the posterior part of the abdomen of hermit crabs, and one on the spiral calcareous dwelling of an annelid. But few sorts are restricted in choice of substrata.

On the hind end of hermit crabs within some snail shells conditions for existence are peculiar, but apparently favorable, as the folliculinids thrive there. Refuse accumulates in the inner end of the snail shell and it may be that bacteria found there aid in the food supply to the folliculinid. Possibly in a similar way the life on the shells of oysters may be profitable to folliculinids since they find an easy food supply of both bacteria and diatoms. Martin (17) and Nelson (19) advance the view that the diatoms and other organisms living in the community upon the oyster shell may serve as food for the oyster, growing there as in a garden to the profit of the oyster. They even suggest that some of the wastage from the oyster going back onto the shell serves to fertilize the crops in the garden. Martin (17) states: "Thus the oyster not only plants a garden upon its shell but fertilizes it."

Folliculinids have their enemies. Various aquatic insects and isopods mow off the tests and make use of them in constructing their own mucous dwellings. But one great drawback to the flourishing of folliculinids is the plugging of their tests with bacterial jelly and burial under accumulations of ooze and sediments. They also may be injured by such unusual parasites as *Pottsia infusorium* Chatton and Lwoff (8) which we observed attacking both *M. andrewsi* and *P.*

amphora from locations north of Baltimore off "Miami Beach", as well as *Platyfolliculina paguri* attached to the abdomen of the hermit crab, *Pagurus pubescens* (3) from Maine.

Some of the empty tests showed that the death of the animal resulted from attack by too many of these parasites. But empty tests arise constantly from the habit of the animal to abandon its test and swim free, in a reduced or larval form. Such free swimmers may add greatly to the plankton. In the Severn R. we took them to the number of 14–100 in a random quart of water (1). A possible utility for human beings may be found in these free swimming folliculinids, for their protoplasm, myonemes and nuclear material all should be good food for whatever animal could sieve them out of the innumerable quarts of water. In the late summer, schools of small young menhaden gyrate through the surface waters with open mouths that take in all sorts of plankton then present, and it is probable that they catch the folliculinids. Thus it may be the menhaden in part that account for the rapid disappearance of folliculinids along the shores of the Chesapeake branches. Eventually it may be proved that the menhaden digest these swimming folliculinids, and that would make the protozoan valuable as contributing to the profit man draws from menhaden fisheries. Again, some of the free swimmers starting from oyster shells might be taken in by oysters and possibly they may contribute to the oyster's food supply, which should endear them to man.

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STUDIES ON THE CHEMICAL PRESERVATION OF WATER SAMPLES*

By

CLAUDE E. ZOBELL and BARBARA FAY BROWN[1]

Scripps Institution of Oceanography
University of California
La Jolla, California

During the storage of water samples collected from the sea, rivers or lakes, increased bacterial activity causes changes in the organic, oxygen, carbon dioxide, ammonia, nitrate or phosphate content or the H-ion concentration of the water (Waksman and Carey, 1935; ZoBell and Anderson, 1936). These changes occur even near 0° C, although refrigeration tends to retard bacterial activity. Consequently, chemical preservatives are frequently added to water samples which are to be analyzed for any of the aforementioned constituents or properties unless the water is to be analyzed soon after collection. Likewise plankton samples are often preserved for later observation.

While the choice of a preservative depends upon the constituent to be determined, in general it should be colorless, miscible with water, chemically inert, preferably volatile or readily neutralized, and of such a nature that it will not interfere with the analytical procedures to be used for the examination of the water. This report is concerned with a study of the relative efficiency of certain preservatives which are widely used or recommended for preventing bacterial activity in samples of sea or fresh water.

EXPERIMENTAL

In the first series of experiments oxygen consumption was used as a criterion of bacterial activity in samples of sea water treated with different preservatives. The work of Bronfenbrenner, Hershey and Doubly (1939) has established that oxygen consumption is a satisfactory method of evaluating the toxic or preservative action of disinfectants. The amount of oxygen consumed is more or less directly proportional to the number of bacteria present and their rate of

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respiration under given conditions (Johnson, 1936). The respiration of bacteria and allied micro-organisms is not influenced by the oxygen tension of the water until it is reduced to less than 0.3 mgm of oxygen per liter (ZoBell, 1940). If the bacteria are not respiring, it is doubtful if they are activating any other chemical changes.

The water samples were collected in 5-gallon carboys, thoroughly shaken to insure uniformity in composition and siphoned into 145 ml glass-stoppered bottles. Preservatives were added to some of the bottles and some were left untreated as controls. Sterile controls were prepared either by heating the water to 124° C for 15 minutes, or by passing it through a fine Mandler filter, after which it was reoxygenated prior to siphoning into glass-stoppered bottles. The dissolved oxygen content of the water was determined by the Winkler method at the beginning of the experiment, and other bottles were analyzed for oxygen after different periods of incubation in the water bath at 22° C. Representative results obtained with some reagents commonly used as preservatives are summarized in Table I.

TABLE I—Oxygen Consumed by Bacteria in 20 Days at 22° C in Sea Water Treated with Different Preservatives

Treatment	*Initial oxygen content*	*Oxygen content after 20 days*	*Oxygen consumed in 20 days*
	mgm/l.	*mgm/l.*	*mgm/l.*
None (control)	7.56	4.41	3.15
0.1% Toluol	7.62	5.16	2.46
0.1% Phenol	7.04	6.30	0.74
0.1% Tricresol	7.59	6.92	0.67
0.1% Formaldehyde	6.69	6.28	0.41
0.5% Chloroform	7.50	7.37	0.13
0.2% Carbon disulfide	7.01	4.34	2.67
1.0% Ether	6.76	3.92	2.83
Heat sterilized	7.36	7.36	0.00
Mandler filtered	7.48	7.45	0.03

The data indicate that none of the reagents in the concentrations used, with the possible exception of chloroform, could be relied upon to preserve samples of sea water for 20 days. However, further experiments have demonstrated that some of the reagents are effective in higher concentrations or for shorter periods of time. This is shown by the data in Table II. Similar results have been obtained with various samples of sea water, as well as with samples of fresh water. The results have been substantiated by experiments in which

bacterial multiplication, nitrate reduction and ammonia production have been employed as criteria of bacterial activity.

DISCUSSION OF RESULTS

Although toluol is commonly used as a preservative for biological materials (Tauber, 1937), it is not satisfactory for the preservation of water samples. Toluol is only slightly soluble in water, but even when its solubility and dispersion have been increased with alcohol or ether it does not prevent the growth of all water bacteria. In fact, certain micro-organisms oxidize toluol. Störmer (1908), Gray and Thornton (1928) and Tausson (1929) have isolated organisms from soil which utilize toluol, and Grant and ZoBell (1942) have reported the occurrence of bacteria in the sea which obtain their carbon and energy requirements from toluol. There was no bacterial activity in water saturated with a mixture of toluol dissolved in chloroform, but the mixture was little more efficacious as a preservative than chloroform alone.

TABLE II—Amount of Oxygen Consumed by Bacteria in Sea Water Enriched with 10 p.p.m. of Peptone after Different Periods of Incubation at 22° C

Treatment	*Oxygen consumed after:*			
	1 day	*2 days*	*5 days*	*10 days*
	mgm/l.	*mgm/l.*	*mgm/l.*	*mgm/l.*
None (control)	2.13	5.70	8.04*	—
0.10% Phenol	0.03	0.38	1.26	5.41
0.25% Phenol	0.00	0.08	0.94	1.60
0.50% Phenol	0.00	0.00	0.00	0.00
0.10% Formaldehyde	0.04	0.36	1.89	4.67
0.25% Formaldehyde	0.00	0.00	0.00	0.00
1.00% Ether	1.72	4.91	7.82*	—
5.00% Ether	0.26	1.07	2.21	4.39
0.50% Chloroform	0.00	0.00	0.07	0.08
0.10% Toluol	0.89	5.27	7.84*	—
0.20% Carbon disulfide	2.29	3.92	5.40	7.86*
0.10% Thymol	1.41	4.38	7.97*	—
Heat sterilized	0.00	0.00	0.00	0.00

* All of the dissolved oxygen was consumed.

One per cent phenol kills most bacteria in a few minutes at room temperature, and 0.1 per cent phenol usually inhibits growth. However, some soil and water bacteria are very resistant to phenol. According to ZoBell, Grant and Haas (1943), a few marine bacteria

have been observed which multiply in the presence of 1 per cent phenol. Some of them multiply in mineral media containing 0.1 per cent phenol as the only energy source. Phenol-resistant or phenol-oxidizing bacteria have been found in soil by Matthews (1924), Gray and Thornton (1928), Evans and Happold (1939), Vigier (1941) and others. Erikson (1941) studied *Micromonospora* species from lake mud which utilize phenol, cresol, toluol, α-naphthol, naphthalene and paradichlorobenzene. We have also encountered phenol-tolerant micro-organisms in sea water, but in about 95 per cent of the 140 ml samples of raw sea water treated with 0.5 per cent phenol there was no evidence of bacterial activity after 20 days at 22° C. Concentrations of phenol as low as 0.05 per cent are definitely inhibitory but are not dependable preservatives. It is doubtful if there are any micro-organisms which can tolerate 5 per cent phenol, but it is hardly practical to add this much to water samples.

None of the cresols used either alone or in combination have proved to be as good as phenol for the preservation of water samples. Pure cresol dissolves in water only with difficulty, and when emulsified with soaps or alkalis, certain salts are precipitated from sea water, thereby rendering the sample useless for many analyses. Certain micro-organisms tolerate a saturated solution of cresol in water. The oxidation of o-cresol, m-cresol and p-cresol by soil micro-organisms has been reported by Gray and Thornton (1928). Cresol-oxidizing bacteria were found in lakes by Erikson (1941). Repeatedly we have found water samples treated with an excess of tricresol becoming soupy with the profuse growth of micro-organisms, although cresol-tolerant organisms do not occur in all water samples.

Some bacteria multiply and are otherwise physiologically active in water treated with 0.1 per cent formaldehyde (Table II). However, in all of the 34 water samples tried, 0.25 per cent formaldehyde has proved to be a dependable preservative. This concentration reduces the solubility of oxygen in water, and formaldehyde may react with nitrogenous organic matter or interfere with chemical analyses which involve the use of oxidizing agents.

Diethyl ether, chloroform and carbon disulfide usually inhibit bacterial activity in water samples, but there are soil and water bacteria which grow in saturated solutions. Chloroform is the most effective of the three. Various combinations of ether, chloroform and carbon disulfide have proved to be no better than when these reagents have been used alone. However, we have found no bacteria which would grow in a combination of 0.25 per cent phenol and 0.5 per cent chloroform. Apparently the phenol-tolerant organisms which are

encountered occasionally cannot tolerate 0.5 per cent chloroform. As much as 0.8 per cent chloroform can be dissolved in water, but it requires considerable shaking of the sample to get 0.5 per cent chloroform in solution. If not completely dissolved, certain colorimetric indicators are preferentially dissolved in the droplets of chloroform, thereby complicating analytical procedures.

Adding enough hydrochloric or sulfuric acid to water to render it more acid in reaction than pH 1.5 provides for its prolonged preservation, as shown by the data in Table III. This requires approximately 5 ml of concentrated HCl or 2.5 ml of H_2SO_4 per liter of sea water. Considerably less acid is required to preserve fresh water, the amount depending primarily upon the buffer capacity. Acidu-

TABLE III—Oxygen Consumed by Bacteria at 22° C in Sea Water Enriched with 10 p.p.m. of Peptone and Treated with Different Concentrations of Hydrochloric or Sulfuric Acid

Acid added	*Amount added*	*pH of water*	*Oxygen consumed after:*		
			5 days	*10 days*	*20 days*
	ml/l.		*mgm/l.*	*mgm/l.*	*mgm/l.*
None	—	8.23	7.81	—	—
Hydrochloric	2.5	1.73	0.95	2.46	5.16
Hydrochloric	5.0	1.38	0.00	0.00	0.04
Hydrochloric	10.0	1.11	0.00	0.00	0.00
Sulfuric	2.5	1.37	0.00	0.08	0.26
Sulfuric	5.0	1.13	0.00	0.02	0.03
Sulfuric	10.0	0.93	0.00	0.00	0.00

lating the water reduces the solubility of oxygen, but the water samples preserved with acid are suitable for most other analyses. Water samples to be analyzed for oxygen content should be treated with the Winkler reagents shortly after the collection of the water. There are a few micro-organisms occurring in nature, such as *Thiobacillus thiooxidans*, for example, which can grow at pH 1.5, but we have never found any evidence of their activities in the numerous samples of sea water, fresh water and sewage which have been acidulated. Phillips and Hatfield (1941) recommend preserving sewage samples for B. O. D. determinations by lowering the pH and then holding the acidulated samples at a low temperature.

Heavy metals such as mercury, copper and silver salts are generally good disinfectants. While low concentrations usually prevent bacterial activity in water samples, salts of heavy metals render the water unsuitable for most kinds of analyses.

Similarly oxidizing agents such as chlorine or iodine compounds,

permanganate or peroxides materially alter the composition of the water and interfere with many analytical procedures. Substances which are effective only in high concentrations dilute the sample too much or may be too expensive to be practical water preservatives.

The use of detergents including 0.1 per cent Aerosol OT, Dreft, Santomerse or Tergitol 4T failed to enhance the preservative action of any of the reagents listed in Table I sufficiently to recommend the addition of detergents. Some of the detergents produced undesirable precipitates in sea water. Petroff and Schain (1940) and others have shown that the killing power of some antiseptics is enhanced by detergents or by surface tension depressants.

Chloroform, formaldehyde or acid are just about as effective in the presence of organic matter as in its absence, so these reagents can be used to preserve plankton samples. There was no bacterial activity in sea water treated with 5 per cent peptone in the presence of 0.25 per cent formaldehyde, 0.5 per cent chloroform or enough acid to reduce the pH to less than 1.5. The acidulation of water does result in the destruction of certain calcareous structures of plankton organisms.

CONCLUSIONS

Toluol, tricresol, thymol, carbon disulfide and diethyl ether are not dependable preservatives for water samples to be examined for constituents or properties which may be altered by bacterial activity.

If they are not objectionable for other reasons, either 0.5 per cent chloroform or 0.25 per cent formaldehyde can be used to preserve water samples.

Nearly all water samples can be preserved indefinitely by the addition of 0.5 per cent phenol, but occasionally micro-organisms are encountered which live in the presence of 1 per cent phenol. When supplemented by 0.5 per cent chloroform, 0.5 per cent phenol is a dependable preservative.

Acidulating water to pH 1.5 which requires about 2.5 ml of sulfuric or 5.0 ml of hydrochloric acid per liter of sea water prevents the activity of micro-organisms ordinarily found in water.

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PLANKTON HARVESTING

By

R. F. SHROPSHIRE

Dow Chemical Company
Wilmington, North Carolina

During the past several years proposals have been made from time to time that new sources of either bulk food material or specialized extracted products could be developed if it were possible to utilize the tremendous amounts of plankton which exist in sea water, thereby increasing the general supply and alleviating certain shortages. In all theories or ideas regarding the utilization of plankton as a source of food, the primary obstacle has been the means of collecting it. Clarke (1) estimates that a townet 2 meters in diameter, towed at a speed of 2 knots, would require a 2½ hour period of filtering to collect the necessary organic matter to maintain a person one day. Hardy (2) thinks of plankton collecting with "huge stow nets" which have openings 18 feet square and bodies of 100 or more feet in length, streamed below an anchored boat or from the anchor chains of buoys. Juday (3), in his discussion of the utilization of aquatic food resources, with particular reference to fresh water plankton, considers collecting with "a large clarifier type of centrifuge at a rate of 1.5 cubic meters in two hours."

During 1942 the writer conducted a series of experiments with a view to designing a device which would permit the collection of plankton in large quantities with economical time and cost factors. At the beginning of the experiments townets, sand filters, flocculation and settling techniques as well as centrifugal devices were tried. These were rejected as impractical for either physical or economic reasons, or both. The requirements of a collecting system should be sturdiness, continuity of operation, relatively low cost from the standpoint of either original installation or maintenance, and the capacity for handling large quantities of sea water in a reasonable length of time.

The device which finally evolved meets most of the above requirements and is shown schematically in Figure 45. Fundamentally it

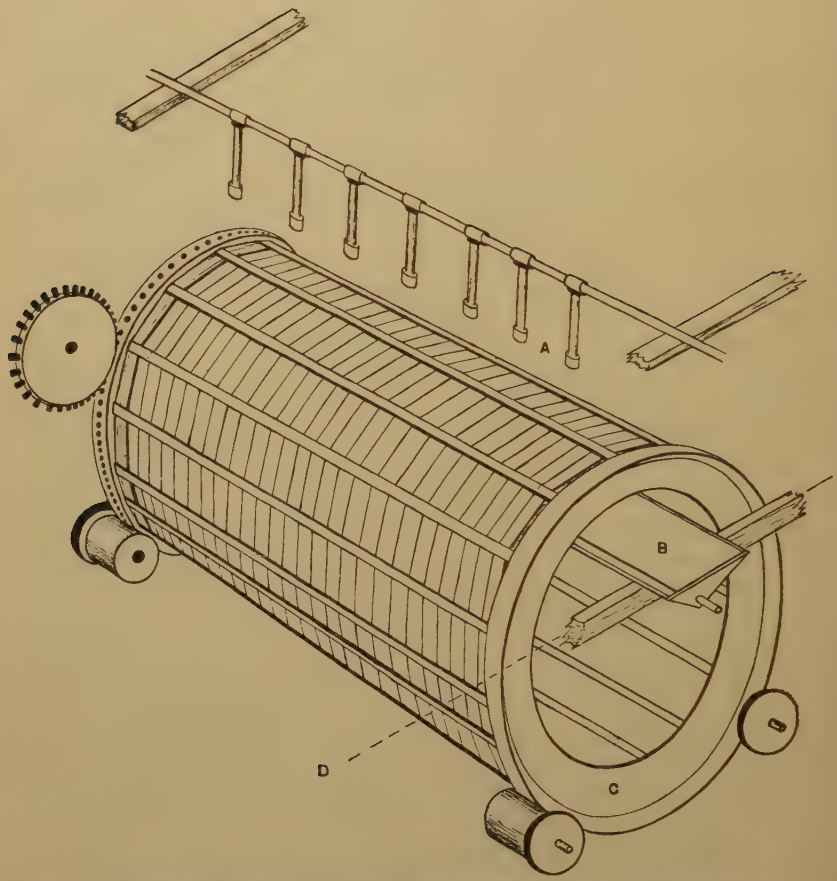

Figure 45. Freehand sketch of plankton harvesting machine. (A) spray nozzles; (B) collecting trough; (C) Flange to prevent backwash; (D) water line. Drawn by Y. H. Olsen.

consists of a large rotary screen, open at one end and closed at the other. Its sides are covered with a screen material. It is placed in the water so that its longitudinal axis is horizontal, and its open end is directed against either the existing current or one that is created artificially. Water entering the open end of the screen passes out through the sides and the plankton in the water stick to the inside of the screening material. By rotating the screen, each successive portion of the rotary screen to which the plankton is adhering is passed beneath a series of inwardly directed sprays. These sprays backwash the screening material into a trough. Various investigators have

estimated that the usual townet used in scientific collecting is reduced to less than one percent of its filtering efficiency at the end of 3 minutes due to the plugging of the meshes by the plankton. This difficulty is obviously avoided in the rotary screen. It is turned at the rate of about one revolution per minute. If the screen is two-thirds submerged, then any given portion of the screen is under water for only 40 seconds, and consequently still has a comparatively high filtering efficiency as it emerges from the water.

Spray nozzles used with the screen were constructed so as to provide a thin flat spray of comparatively high velocity and low volume. A collar-like baffle reduced the aperture of the open end of the screen and prevented the entering water from washing back out. When operated in a current or a tideway, the only power used in the experimental models constructed so far was that required to rotate the screen and to drive the pump supplying water to the spray nozzles. It is conceivable that the use of a helical fin on the outside of the screen could eliminate even the power required to rotate it.

The first model rotary screen which the writer constructed was 4 feet in diameter and 4 feet long. It was covered with No. 15 triple X bolting cloth having 150 meshes per inch. Volumes of water up to 5,000 gallons per minute with a gradient of less than 1 inch through the screen were handled. The screening material was sewn to cotton tapes to form panels 48 inches long and 10 inches wide. The cotton tapes were used to fasten the panels to the frame work of the screen itself. As an added strengthening feature, linen cord was wrapped around the screen over the silk, successive turns being spaced from 1¼ inches to 1½ inches apart. The screen was supported upon two trunions at each end and rotated by a sprocket which engaged in a ring fastened around one end. The screen was operated first in a brine canal having a velocity of about 2 feet per second. The screen with its mountings and spray nozzles was installed subsequently in a large wooden box which was provided with suitable weirs and manometers. The water that was filtered was pumped into this box in order to obtain accurate quantitative data on the amount of water filtered by the screen, and to estimate the efficiency of the screen as a filtering device. Table I shows the results of actual tests of the operation of this model.

A second model, 8 feet in diameter and 16 feet long, was constructed and suspended in a well in a large float. It was operated in a tideway and was provided with a motor-driven propeller at the mouth of the screen which forced water into it during that portion of the day when there was inadequate tidal flow. It was estimated that this 8 x 16

TABLE I—Plankton Collection Conditions and Yields

Filter Run No.	Sept. 1942	Temperature Sea	Temperature Air	Wind Direction	Wind Force	State	Sea Degree	Sea Direction
24	4	25.5	26.0	NE	0–1	Smooth	0–1	E
25	4	25.6	27.2	N	0–1	Smooth	0–1	NE
26	8	25.2	26.4	NW	0–1	Smooth	0	—
27	9	25.5	26.0	ENE	0–1	Smooth	0–1	—
28	10	25.4	26.0	W	0–1	Smooth	0–1	W
32	14	25.6	27.0	W	0–1	Smooth	0–1	W

* These data were obtained when the screen was operated in conjunction with a pump having a rated capacity of a thousand gallons per minute.

Tide Condition	Sky Condition	Sky Type	Sky Extent	Total Weight Flow Gallons*	Total Weight Wet Cake in grams
Flooding	Overcast	Cumulus	4	32384	1021
Flooding	Overcast	Cumulus	8–9	32384	1214
Ebbing	Cloudy	Cumulo-stratus	4	48576	1279
Ebbing	Cloudy	0		23074	2546
Ebbing	Cloudy	0		12144	1529
Flooding	Cloudy	Cumulus	1–2	16192	1474

foot screen had a filtering capacity on the order of 25,000 gallons of water per minute, when properly backwashed and rotated at a rate of one revolution per minute. Spray nozzles were spaced about 9 inches apart so that the fan-shaped sprays from individual nozzles slightly overlapped. Pressure to the spray nozzles was about 45 pounds per square inch. Flow through the individual nozzles was approximately 2 quarts per nozzle per minute.

A second screen 4 feet in diameter and only 15 inches long provided with two spray nozzles was used in tandem with the 8 x 16 screen, thus providing a second concentrating step. The almost gelatinous material coming from the trough of the second concentrating screen was pumped through a 12 plate filter press, each plate having an area of 144 square inches. Cake from the filter press was subsequently treated chemically.

It is believed that a device such as this rotary screen provides a practical means of harvesting plankton economically.

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A PRELIMINARY REPORT ON THE FOULING CHARACTERISTICS OF PONCE DE LEON TIDAL INLET, DAYTONA BEACH, FLORIDA

By

B. R. RICHARDS and WILLIAM F. CLAPP[1]

William F. Clapp Laboratories, Inc.
Duxbury, Massachusetts

INTRODUCTION

In December 1941, the writers visited the exposure location of the North Florida Test Service on Ponce de Leon Inlet, near Daytona Beach, Florida, for the purpose of examining the extent of borer attack in this locality, and to determine the station's suitability for exposure tests on treated piling and for tests in the related field of marine fouling. Arrangements were effected for exposing a series of gum pine blocks throughout the year 1942 to record the progressive borer attack, and simultaneously, to record the growth of all marine fouling organisms prevalent at this location.

The exposure plan required immersion of 13 untreated wood blocks 6″ x 8″ x ¾″, marked 0, 1–12, on January 1, 1942. The blocks were nailed to a supporting two-by-four and submerged in the same manner as their paint test panels—2 feet below the low-tide line.

On the first of February the blocks marked "0" (control) and "1" were removed, packed in wet newspaper, and shipped to Duxbury for detailed examination; at the same time a new "0" control block was inserted in the rack. Thus, each month a control block would be obtained which represented growths that accumulated in the previous month and a numbered block which represented the accumulated growth from January 1, 1942 to the date of its removal. This plan was successfully followed throughout 1942.

The present report is based only on the monthly "control" blocks. Conclusions drawn from the continued exposures will be presented in a later contribution.

[1] The co-operative assistance of Messrs. E. L. Messler and G. C. Quelch of the North Florida Test Service, and of Dr. G. H. Young, of the Mellon Institute of Industrial Research, who effected the necessary arrangements for the study, is gratefully acknowledged.

EXPOSURE SITE DATA

The marine exposure facilities of the North Florida Test Service are located on the north shore of Ponce de Leon Inlet, one-fourth of a mile from the Atlantic Ocean. The flow of water past the piers, from which the racks of panels are suspended, is decidedly strong, the direction of flow depending on the tide. The piers extend out into the water for approximately 60 feet and are L shaped; from the heads of these the total immersion racks are suspended in water which is 8 to 10 feet deep at low tide. The total immersion panels are suspended 1–2 feet below the low water mark. Intertidal panels are at the median tide line. The tide averages about 4 feet at this location. Both total immersion and intertidal panels are hung in wooden frames consisting of grooved 2″ x 4″ wood lapped and bolted at the four corners. The panels, six to eight on a rack depending on their size, are mounted in the rack edge to edge, but not touching each other. The surfaces of the panels are exposed to the sweep of the current, since the racks are hung in the same direction as the current. There is no chance of dead water forming between panels hung in this manner, as may be the case when panels are suspended from a rack with the surfaces of the panels facing each other. The racks are suspended from the pier by brass chain.

The general fouling picture at this location is impressive, not only in types of organisms, but also in intensity. Barnacles and the filamentous bryozoans, *Bugula*, are the predominant forms. Calcareous tube worms, hydroids, encrusting bryozoans, mollusks (oysters and clams) and algae are also found in considerable numbers. Several species of Teredinidae are very prevalent. As a rule these organisms are very destructive in timbers where fouling organisms thrive—in which respect the Daytona location seems to be typical. The fouling intensity and species show seasonal fluctuations; however, an unprotected panel immersed at this site for at least two months at any time of the year is heavily fouled at the end of the two-month period.

The conventional meteorological data for the test site are recorded in Table I.

TABLE I—Meteorological Data

Average wind velocity	9 mph. southeast	
Annual rainfall:	47.25 inches	
Annual total sunlight:	240 days	
Percentage of theoretical total sunlight:	69%	
Average relative humidity:	86% at 7:30 A. M. 54% at 12:00 M.	
Maximum current velocity:	4 mph	
Mean average tide:	3.8 feet	
	1941	1942
Average water temperature:	70° F	69° F
Maximum recorded water temperature:	84° F	81° F
Minimum recorded water temperature:	54° F	58° F

RESULTS OF THE SURVEY

For purposes of this report the detailed memoranda describing the incidence of the various identifiable organisms on the test blocks have been summarized in the following tables. Table II shows the borer

TABLE II—Monthly Borer Attack in Control Panels in 1942

Month	*Water Temp. on 20th in °F*	*Total No. of* Teredinidae	*Size Range in mm*	*Total No. of* Pholadidae	*Total No. Limnoria lignorum* White
January	68	0	——	0	18
February	61	0	——	0	4
March	58	5*	min.	0	51
April	60	11	min.	0	131
May	67	960*	min. to 40	0	81
June	72	250*	min. to 30	0	40
July	78	5*	min. to 15	0	132
August	76	0	——	0	v
September	81	250*	min. to 40	v**	25
October	76	5*	min.	0	50
November	72	24*	min. to 5	0	57
December	66	5	min.	0	101

Key: 0 Organism absent.
* Identified specimens of *Bankia gouldi* Bartsch included in totals.
** Identified specimens of *Martesia striata* L. included.
v Organism present, but no count available.
min. Minute.

attack; Table III the attack by fouling organisms. It should be noted that these summaries were compiled from the *control* blocks only, and thus represent the incidence and growth in each month, *independent* of the previous month.

DATA ON BORER ATTACK

In general, the data in Table II are self-explanatory, and need no comment. Seasonal effects for the Teredinidae are obvious. It appears that the 1942 periods of heaviest attack were May–June, and September.

There appears to be more intense *Limnoria* infestation in the period April through July. However, the *Limnoria* attack is not of sufficient intensity to enable positive conclusions to be drawn concerning seasonal variations. The figures may well represent the expected variation in *Limnoria* counts on single test panels.

DATA ON FOULING ORGANISMS

The data in Table III are sufficiently detailed as to need little comment. Seasonal effects are again obvious. Thus, there appears

TABLE III—Monthly Fouling Incidence in 1942

Month	Water Temp. on 20th in °F	CRUSTACEA—CLASS CIRRIPEDIA: Total No. of Balanus	Size Range in mm	Balanus improvisus Darwin	Balanus amphitrite Darwin	Balanus eburneus Gould
January	68	7200	min. to 3	0	0	0
February	61	7440	min. to 4	z	z	0
March	58	1565	min. to 4	z	z	0
April	60	605	embryonic	0	0	0
May	67	175§	juv. to 7	0	z	z
June	72	250§	3 to 7	0	0	z
July	78	250§	1 to 3	0	z	0
August	76	6500§	1 to 3	0	0	0
September	81	800§	3 to 10	z	z	z
October	76	125§	juv. to 5	0	0	z
November	72	6500§	juv. to 4	0	0	0
December	66	250§	juvenile	0	0	0

TABLE III—Monthly Fouling Incidence in 1942 (cont.)

Month	Water Temp. on 20th in °F	BRYOZOA: Bugula sp.	Bugula flabellata (Thompson)	Bugula neritina (L.)	Electra crustulenta (Pallas)	Electra monostachys (Busk)	Schizoporella unicornis (Johnston)	Cryptosula pallasiana (Moll)
January	68	0	0	0	0	0	0	0
February	61	0	0	0	0	0	0	0
March	58	5	0	4	2	0	1	2
April	60	30	5	41	num.	0	many	few
May	67	many	0	many	num.	0	num.	0
June	72	many	0	many	0	0	few	0
July	78	0	0	40	0	1	many	0
August	76	0	0	many	0	0	few	0
September	81	few	few	many	many	0	many	many
October	76	many	many	many	few	0	many	many
November	72	few	few	many	0	0	many	many
December	66	many	many	many	0	0	0	0

TABLE III—Monthly Fouling Incidence in 1942 (cont.)

Month	Water Temp. on 20th in °F	MOLLUSCA: Anomia simplex D'Orbigny	Mytilus hamatus Say	Ostrea virginica Gmelin	ANNELIDA Serpulidae	COELENTERATA Tubularia sp.	CHORDATA Tunicata	Silt
January	68	0	few	0	0	9	0	mod.
February	61	0	0	0	0	10	0	trace
March	58	0	0	0	2	trace	1	0
April	60	few	0	0	0	13	1	light
May	67	many	few	0	many	trace	0	trace
June	72	hundreds	0	hundreds	hundreds	0	0	trace
July	78	0	0	0	num.	trace	0	light
August	76	0	0	0	many	0	0	trace
September	81	0	0	0	many	0	0	trace
October	76	0	0	0	0	trace	0	trace
November	72	0	0	0	0	light	0	light
December	66	0	0	0	few	light	0	light

Key: 0 Organism absent.
z specimens identified.
§ count approximated.

trace or few 1–15 specimens.
moderate or numerous 16–40 specimens.
heavy or many 41–100 specimens.

TABLE IV—Comparative Intensity of Monthly Fouling Attachment

Month	Teredinidae	Pholadidae	*Limnoria lignorum* White	*Balanus*	*Other Mollusca*	*Bryozoa*		*Annelida*	*Hydrozoa*	*Tunicata*	*Silt*	*Misc.**
						Encrusting	*Filamentous*					
January	0	0	x	xxxxx	x	0	0	0	x	0	xx	x
February	0	0	x	xxxxx	0	0	0	0	x	0	x	xxx
March	x	0	xx	xxxxx	0	x	x	x	x	x	0	xxx
April	x	0	xxxx	xxxxx	x	xxx	xx	0	x	x	x	x
May	xxxxx	0	xxx	xxxx	xxx	xx	xxx	xxx	x	0	x	x
June	xxxx	0	xxx	xxxx	xxxx	x	xxx	xxxx	0	0	x	x
July	x	0	xxxx	xxxx	0	xxx	xxx	xx	x	0	x	xx
August	0	0	x	xxxxx	0	x	xxx	xxx	0	0	x	0
September	xxxx	x	x	xxxxx	0	xxx	xxx	xxx	0	0	x	xxx
October	x	0	xx	xxxx	0	xxx	xxx	0	x	0	x	xx
November	x	0	xx	xxxxx	0	xxx	xxx	0	x	0	x	xxx
December	x	0	xxx	xxxx	0	0	xxx	x	x	0	x	x

Key: 0 Organism absent. (x) Organisms per panel: 1–30; (xx) 31–60; (xxx) 61–100; (xxxx) 101–500; (xxxxx) 501–up.

* This group includes shrimp, starfish, flatworms etc. which enter fouled regions on the panels as mature, motile organisms.

to be a well-defined growth peak for the Cirripedia (*Balanus*) beginning in November and extending through February. A secondary set apparently took place in August.

There appear to be two seasonal peaks for the bryozoans: one is the period May through July; the second is the period September through November, and into December for certain of the species. Mollusks are apparent only during May and June. Annelids (tube-worms) are very prevalent in the period May through September.

Dwarf hydroids (Tubularia) are present only during the period October through April. However, the active presence of these growths during the summer months has been demonstrated further north, at Wilmington, North Carolina. This suggests that summer water temperatures at Daytona may be too high for hydroid survival there.

The data in Tables II and III have been condensed in Table IV, and are shown schematically in order to facilitate comparisons of growth intensity and uniformity. It is apparent that the heaviest and most uniform fouling is by *Balanus*, as would be expected. The next most prevalent organisms are filamentous bryozoans which are rather uniformly present from March through December. Encrusting bryozoans are also present for the greater part of the year.

SUMMARY

A preliminary fouling and borer survey of the North Florida Test Service marine exposure location has demonstrated the active presence of at least three identified species of *Balanus*, six identified species of bryozoans—both encrusting and filamentous, three identified species of mollusks, of annelids, and of dwarf hydroids. At least three types of boring organisms are actively present, of which the very destructive and rapidly growing *Bankia gouldi* (Bartsch) is the most prevalent. (Attack by this organism resulted in complete destruction of continuously-exposed test boards in 8 months.)

While the expected seasonal variations in intensity are found, the general growth picture is one insuring fouling conditions during every month of the year—a desirable condition for exposure test purposes. The station appears to offer an excellent compromise between middle Atlantic and semitropical conditions as well as a desirable location from the standpoint of marine testing.

Studies of this marine location are being continued and detailed findings will be reported subsequently.

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A THEORY OF SURFACE WATER MOTION DEDUCED FROM THE WIND-INDUCED MOTION OF THE PHYSALIA*

By

ALFRED H. WOODCOCK

Woods Hole Oceanographic Institution
Woods Hole, Mass.

For many years the author has wondered why the Portuguese Man-of-war (*Physalia pelagica* Bosc.) is so oriented physically that it is consistently driven by the wind about forty-five degrees to the left of the direction in which the wind is blowing[1] (Figure 46, left). Records kept of this leftward tendency showed little variation in distribution or time, within the limits shown in Table I. In the literature concerning this animal no explicit reference to this question of its motion relative to the wind and water has been found.

Physalia is a jellyfish of the order siphonophora which lives in the warm surface waters of the oceans. For the purposes of this discussion, its body may be considered as having two parts: the upper part a balloon-like float called a pneumatophore which is on the surface of the water, and the lower part a mass of tentacles which are attached to the ventral lee side (i. e., side opposite to direction of motion) of the float (Figure 46). Small fish and plankton, upon contact with these tentacles, are stunned by powerful stinging organs after which they are hoisted to the digestive zooids. During calm weather the tentacles may extend many meters below the sea surface, but when driven along by even a moderate wind these tentacles stream out behind the float with only a few centimeters of vertical extension. This means that the Physalia would necessarily "fish" through a relatively shallow layer near the surface while sailing. However, the large volume of water passing its tentacles when it is driven along by the wind should more than compensate for the lack of depth in its "fishing."

In speculating about reasonable explanations for this animal's persistent leftward motion, it was thought probable that the answer

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[1] Hereafter, in all references to directions of motion on or within the surface waters concerned it will be assumed that the observer is facing in the direction toward which the wind is blowing.

Figure 46. Laboratory photograph showing Physalia in sailing position, with tentacles streaming behind. For convenience, a mirror image of the northern hemisphere animal is used to represent one from the southern hemisphere. The rods indicate wind direction; arrows show resulting direction of motion: (left) in northern hemisphere and (right) in southern hemisphere.

TABLE I—Physalia Observations at Sea

Number of Physalia seen moving to:		*Date*	*Wind Force*	*Lat. N.*	*Long. W.*
*Left**	*Right**				
				North Atlantic	
2	0	Oct. 6, '39	3	39°22′	70°45′
1	0	Aug. 13, '40	–	39 23	72 28
1	0	Aug. 13, '40	–	39 23	72 28
13	0	Apr. 15, '41	4	33 31	74 52
1	0	Apr. 16, '41	3	29 26	74 42
1	0	Apr. 17, '41	3	25 29	72 24
2	0	June 28, '41	4	39 00	46 00
6	0	July 7, '41	4	39 00	46 00
2	0	Aug. 8, '41	2	40 18	67 21
3	0	Aug. 10, '41	5	36 26	64 08
34	0	Mar. 16, '42	3	26 36	80 00
11	0	Mar. 23, '42	5	25 42	80 06
				Gulf of Mexico	
113	0	Mar. 26, '42	5	26 27	84 25
89	0	Mar. 26, '42	4	26 42	84 47
76	0	Mar. 26, '42	4	27 00	85 10
37	8	Apr. 27, '42	4	27 29	86 35
29	1	Apr. 28, '42	5	27 55	89 32
421	9	Totals			

* Left and right of wind as illustrated in Figure 46.

would concern factors of survival such as the catching of food or the avoiding of danger.

In sections of the North Atlantic where the sargasso weed is common, the Portuguese Man-of-war is sometimes seen with tentacles hopelessly and fatally entangled in the weed. Since the weed is most often seen in lines formed parallel with the wind, it was thought possible that some characteristic of the motions of the surface water might cause a leftward-moving animal to avoid more of these lines of weed than a rightward-moving animal.

Langmuir (1938) made a study of formations of lines of drift in fresh water and found that these lines marked zones where surface waters are converging and sinking. "Midway between adjacent streaks" he found subsurface waters rising and diverging laterally (Figure 47A & B), so that under the action of winds the surface water was moving in a series of parallel left and right helical vortex pairs.

From Langmuir's measurements it became clear that the lines of sargasso weed, seen during winds on the sea surface, were collected in the convergent zones of similar vortex circulations. Thus, it was apparent that a Physalia sailing straight downwind would stay within a convergent zone and there encounter a maximum amount of entangling weed or other surface drift. However, this did not help in solving the problem of the animal's persistent leftward motion, for

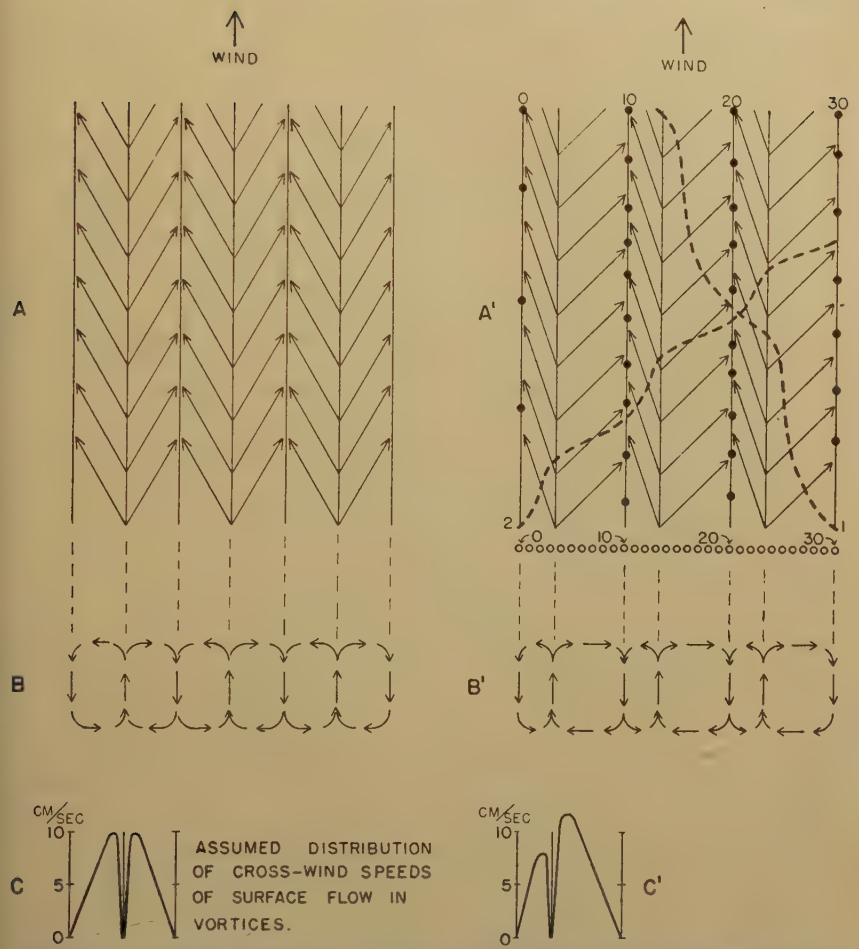

Figure 47. Idealized drawings of wind-induced helical motions in surface waters, with an illustration of the possible effect of asymmetrical vortices upon the drift of bottles and of Physalia. (See text for further explanation.)

a motion either to the left or to the right of the downwind direction, sufficient to oppose the converging currents, would make it possible for a Portuguese Man-of-war to avoid remaining within a convergent zone. There appeared to be no way in which a Physalia moving to the left across a vortex field (Figure 47A) would pass through fewer convergences than an animal moving to the right.

What might be the effect of the vortices upon the feeding of the Physalia, or upon the distribution of its food?

Langmuir's measurements of ascending rates in the upwelling divergences in fresh water give values (1 to 1.5 cm/sec) which are well in excess of the settling rates of at least one very abundant zooplankton, *Calanus finmarchicus* (Gunnerus), (Gross and Raymont, 1942). In the ocean these rates of ascent in divergences would be greater than in fresh water, under otherwise similar conditions, since salt concentrations due to evaporation should cause a general increase in the rates of vertical motion. Hence it seems probable that the upwelling divergent zones at the sea surface contain plankton carried up from the bottom of the mixed layer. When within the regions of lateral surface flow from the divergences to the convergences, the suspended plankton would have ample time to settle many centimeters below the reach of the Physalia's tentacles before descending in a convergent zone. For the Physalia, this would mean that surface waters would be divided into rich and poor feeding bands extending parallel with the wind, the divergent zone being rich and the convergent zone being poor. However, there still appeared to be no reason why a Physalia moving forty-five degrees to the right of the downwind direction across a field of symmetrical vortices (Figure 47A) would not fare as well as one moving forty-five degrees to the left.

A possible indication of greater advantage from leftward motion seemed to lie in the assumption of an asymmetrical arrangement of the wind-induced parallel vortex pairs, with the larger eddy rotating clockwise (Figure 47A′ & B′). Such an asymmetry would imply an unequal distribution of the forces affecting the circulations. The deflecting force of the earth's rotation (Corioli's force) might supply this inequality.

If Corioli's force is assumed to cause an asymmetry of the wind-induced vortices, and if this asymmetry presents an adequate indirect reason for the leftward motion of the Physalia, it then seemed obvious that a simple first check upon the validity of these assumptions could be made from observations of the Portuguese Man-of-war in the southern hemisphere. If the above assumptions were correct, then in southern latitudes one would expect to find most of the Physalia moving to the right of the wind. This expectation proved to be correct concerning specimens available for examination from the southern hemisphere. Of twenty-two south-latitude animals preserved at the Museum of Comparative Zoology, Harvard University and at the U. S. National Museum in Washington, nineteen were left-handed and three were right-handed. That is, eighty-six per cent of them were mirror images of the majority of the animals from the northern hemisphere, and, as a result, would sail to the right of the

wind. This partly-confirmed prediction suggested that the assumptions concerning the effect of Corioli's force upon the vortices might have value in fact.

DRIFT BOTTLE EXPERIMENTS

Some recent drift bottle experiments give further evidence that the assumption of asymmetry in the vortices, based upon deductions drawn from the Physalia movements, may be correct.

During April 1942, some special drift bottle tests were made in the surface waters of the Central Sea area of the Gulf of Mexico (Lat. 27° 30′ N., Long. 86° 40′ W.). One hundred numbered bottles (130 cc displacement 12 cm draft) with hard rubber caps were painted a bright orange color and then ballasted with water so that each bottle had a buoyancy of one gram (± 0.1 gram) in the sea water in which they were used. With this small buoyancy the tops of the bottles floated so near the water surface that the surface film swept across the tops continuously. Thus direct wind drag on the bottles was reduced to a minimum.

Before distributing these drift bottles on the sea surface it was necessary to be free of water disturbances caused by the hull of the research vessel ATLANTIS. For this purpose a twelve foot dinghy was used, but this practice necessarily confined these drift studies to winds below Beaufort 5.

Figure 47A′ gives an example of one orientation of the bottles relative to the wind. The open circles show how the bottles were initially distributed from the small boat. Within three to five minutes all of these bottles were always reformed into new lines parallel with the wind direction (shown by the black circles) and at right angles to the initial line of distribution. From Langmuir's measurements (1938, p. 2), and from later tests made by the author, it has been found that the surface water in the convergences moves downwind more rapidly than the water between them. Hence, one would expect that the bottles which happened to be dropped into the water nearest to the convergences would be the lead bottles in the line (i. e., first at the downwind end of the line). With these bottle numbers as references and with the known numerical order and spacing of the preceding and succeeding bottles as initially distributed, one could observe the number of bottles in any particular convergence which came from the right and left of that line, hence the comparative distances to the two adjacent divergences. For example, bottle number twenty in figure 47A′ (open circles) was dropped in a convergence and became lead bottle in that line. If the helical vortex

pairs were perfectly symmetrical, then one would expect to find bottles fifteen to twenty-five in the line, with number twenty leading. If the vortices are asymmetrical, with the clockwise eddy larger, then number twenty would be followed by more numbers less than twenty and fewer numbers greater than twenty. In eighteen series of such drift tests (Table II) made in deep water in the Gulf of Mexico, it was found that an average of six bottles came from the left of the line compared to four from the right. Three of these tests showed a small opposite tendency.

No exact method could be devised to make the bottle spacing equidistant initially. However, by tossing them over rhythmically, about two meters apart, the dinghy being rowed steadily at right angles to the wind direction, it was thought that any errors due to spacing would be random. Table II gives the data relative to these drift bottle tests.

TABLE II—Drift Bottle Tests in the Gulf of Mexico (27° N. 86° W.) in 1942

Test No.	*Date*	*Number and Percentage of bottles originating:*				*Temperatures in (°F)*			*Wind*		*Depth of mixed layer in meters*
		*on left**		*on right**		*Air*		*Water*	*Force*	*Dir.*	
	1942	*No.*	*%*	*No.*	*%*	*Wet*	*Dry*			*(true)*	
1	Apr. 21	38	46	44	54	67.7	70.9	71.8	2	63°	7
2	Apr. 21	45	58	33	42	67.0	71.1	72.4	2	85	
3	Apr. 22	46	54	39	46	64.2	73.1	75.5	2	25	
4	Apr. 22	60	74	21	26	64.4	71.6	73.7	3	15	11
5	Apr. 22	46	55	38	45	64.4	71.5	73.7	3	25	
6	Apr. 23	61	69	27	31	67.4	71.4	70.5	3	95	
7	Apr. 23	60	72	23	28	67.8	71.8	70.6	3	100	
8	Apr. 23	56	69	25	31	67.8	71.8	70.6	3	100	9
9	Apr. 23	48	60	32	40	67.6	71.8	70.8	3	100	to
10	Apr. 23	36	47	41	53	66.8	71.9	70.9	3–4	95	15
11	Apr. 23	49	61	31	39	66.5	72.1	71.0	3	95	
12	Apr. 23	46	60	32	40	66.5	72.1	71.0	3	90	
13	Apr. 25	40	51	39	49	67.7	71.7	70.1	4	105	27
14	Apr. 26	51	63	30	37	67.3	71.5	70.2	3	115	20
15	Apr. 27	41	56	32	44	68.5	72.4	71.4	2.5	105	
16	Apr. 27	52	71	21	29	68.2	72.2	71.4	2.5	105	4
17	Apr. 27	32	44	40	56	68.3	72.2	71.5	2	105	
18	Apr. 27	50	74	18	26	68.7	72.6	71.8	2.5	110	
Average percent			60.2		39.8						

* Facing downwind.

DISCUSSION

Figure 47A gives an idealized drawing of the surface motions in paired symmetrical helical vortices uninfluenced by the deflectional effects of the earth's rotation. Figure 47A′ shows a hypothetical relative directional change of the surface motions under the influence

of the deflection effect. The total deflectional displacement of motion in a given vortex pair is probably affected by their unknown subsurface motions. However, the general effect of the surface deflections should be an extension of the axial component of motion in the counterclockwise vortex and a shortening of the axial component in the clockwise vortex. These changes in the axial component should result in a retarding of the rate of cross-wind flow in the left vortex and an increase in the cross-wind flow in the right (clockwise) vortex (Figure 47C′). Thus the clockwise vortex should be larger and the counterclockwise vortex smaller; of course the total energy of motion of each vortex pair would remain the same.

One important result of such a distortion of the surface vortices, as it might affect the feeding of the Physalia, would be the reduction of the speed of cross-wind flow in the counterclockwise vortex and the increase in the speed of cross-wind flow in the clockwise vortex. A decrease in the rate of this cross-wind flow from a point near the divergence to the zero point in the convergence would be important also (Figure 47C′). The average value of the cross-wind flow in this figure is based upon observed rates of drift-bottle convergence, and the slope of the curves seem reasonable in view of the probable cumulative effect of wind-mixing in retarding the surface water while it is moving from the upwelling to the convergence. In such a system of surface flow a Physalia moving to the left would remain, in a given time, within rich waters longer than one moving to the right (Figure 47A′, dashed lines). Also, the Physalia moving to the left would pass through fewer convergent zones, as a consequence of its more extended course diversions between these zones (Figure 47A′, dashed line 1). Thus in the northern hemisphere the Physalia moving to the left should catch more food at less risk of fatal entanglement than one moving to the right.

Hence, it appears that the circulation of surface waters as outlined above would explain the motions of the drift bottles as well as the proponderance of motion of the Physalia to the left of the downwind direction.[2] Further drift bottle tests should soon give conclusive data concerning the reliability of the results obtained from these first, rather crude, exploratory tests. Plankton net tows in convergent and divergent zones should readily show the effects of the helical vortices upon plankton population distribution in surface waters. Also,

[2] Large numbers of *Vellela* were recently seen in the Gulf of Mexico. Of hundreds of animals observed, all were sailing to the left of the downwind direction. This seems to indicate that the factors which produce the leftward motion under the influence of the wind are not confined to the Physalia alone.

further observations of Physalia north and south of the equator may enlarge upon the biological implications of this apparent reversal of asymmetry with hemisphere.

SUMMARY

Observations are given which show a curious tendency of the pneumatophore of the Portuguese Man-of-war (*Physalia pelagica*) to be so oriented with the wind as to cause the animal to be driven about forty-five degrees to the left of the downwind direction. These observations were made over a large part of the North Atlantic and Gulf of Mexico during a period of about three years.

A hypothesis advanced to explain this leftward tendency required in part that: (a) southern hemisphere *Physalia* be mirror images of those in the northern hemisphere, and that they sail to the right of the downwind direction: (b) wind-induced helical vortex pairs be set up in surface waters at sea, and that these vortices be asymmetrical.

Of twenty-two Physalia from the southern hemisphere available for examination in museum collections, nineteen (86%) were mirror images of those in the northern hemisphere and would sail to the right of the wind.

Drift bottle tests on the open sea have shown that lines of convergence are set up by the wind which are oriented parallel with the wind direction. These lines apparently mark the surface convergences of parallel left and right helical vortex pairs set up by the wind. The tests suggest that these vortices were asymmetrical, the larger vortex of each pair rotating clockwise, the smaller counterclockwise (facing downwind). This asymmetry was shown by the larger number of bottles drifting into the average convergence from the left of that line, the initial distribution of the drift bottles having been approximately equidistant in a line at right angles to the convergence lines.

Such an asymmetry would imply an unequal distribution of the forces driving the circulations. The deflecting force of the earth's rotation may supply this inequality. Hence the following theory is proposed: Corioli's force and frictional forces may produce an asymmetry in the wind-induced helical vortex pairs developed in surface waters on the open sea.

Four facts are apparent: first, the widespread prevalence of the leftward motion (in the given areas) of the animals; second, the reversal of asymmetry among *Physalia* of the southern hemisphere; third, the formation of lines of convergence in the surface water of the open sea parallel with the wind direction; fourth, the six to four average relationship between the number of drift bottles which came

from the left of the surface convergences as compared to those coming from the right of the convergences. While the eighteen drift tests recorded in Table II do not conclusively prove an asymmetry in the helical vortex as developed in the surface waters of the deep sea, they are highly suggestive that such asymmetry exists. Further drift bottle tests and other experiments are planned, but in view of the inherent uncertainties of these times it seems wise to publish these results now.

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THE VERTICAL STABILITY OF THE COAST AT BOSTON IN THE LIGHT OF RECENT TIDE OBSERVATIONS

By

H. A. MARMER

U. S. Coast and Geodetic Survey
Washington, D. C.

Since August 1921 the U. S. Coast and Geodetic Survey has had an automatic tide gauge in operation in Boston Harbor. Periodically the elevation of the tide staff, to which the observations are referred, has been checked by leveling it to a number of bench marks. These observations now cover a little over 22 years and furnish definite data for use in the study of the vertical stability of the coast.

The automatic tide gauge furnishes a record in the form of a continuous curve of the rise and fall of the tide, drawn to a definite scale. In tabulating this record it is customary to tabulate the times and heights of each high and low water, as well as the height of the tide at the beginning of each hour of the day. The heights are referred to a fixed tide staff, the zero of which is set at some convenient elevation so that the low waters will not fall below it.

The heights derived from such tabulations for high water, sea level or low water from any series of observations are thus relative heights—relative to the elevation of the tide staff used. The essential point is that the zero of the tide staff be kept at a constant elevation. If any change in the elevation of the tide staff occurs at any time, the exact amount of change must be determined accurately, so that the whole series may be referred to a staff with a constant elevation. This purpose is served by periodic leveling between tide staff and bench marks.

In the study of the vertical stability of the coast, any one of the three tidal phases, namely high water, sea level or low water may be used. However, the sea level data are the most satisfactory; for as we shall see, high water and low water are subject to variations from which sea level is free. In Table I the yearly average values of high water, sea level and low water are given in feet with reference to the tide staff of constant elevation. For convenience the data are arranged in two groups of eleven years each.

An examination of the table shows that high water, sea level or low water may differ by as much as two tenths of a foot from year to year.

TABLE I—Yearly Average Heights, Boston, Mass.

Year	H. W.	S. L.	L. W.	Year	H. W.	S. L.	L. W.
1922	12.80	8.09	3.12	1933	12.83	8.30	3.52
1923	12.79	8.08	3.11	1934	12.63	8.12	3.36
1924	12.83	8.10	3.14	1935	12.79	8.25	3.46
1925	12.70	8.03	3.11	1936	12.82	8.19	3.32
1926	12.70	8.06	3.18	1937	12.93	8.28	3.37
1927	12.80	8.17	3.30	1938	13.00	8.32	3.37
1928	12.66	8.07	3.25	1939	13.05	8.32	3.34
1929	12.63	8.09	3.33	1940	13.07	8.34	3.32
1930	12.54	8.08	3.39	1941	13.04	8.35	3.40
1931	12.76	8.24	3.49	1942	13.09	8.39	3.45
1932	12.74	8.22	3.46	1943	13.06	8.34	3.37
Means	*12.72*	*8.11*	*3.26*		*12.94*	*8.29*	*3.39*

For a given year the heights of any one of these tidal datums may be higher or lower than the preceding year, but it is evident that in general there has been a more or less progressive increase in height. Considering the means of the two eleven year groups, high water has increased 0.22 of a foot, sea level 0.18 and low water 0.13. The changes in height from year to year, and their relations as betweeen the different datums, become clearer if the data of the table are represented graphically as in Figure 48. The horizontal line associated with each of the diagrams represents the average value of the particular datum for the 22 years of observations. For convenience the yearly values for each datum are given with reference to the average value, rather than with reference to the tide staff. The significance of the light sloping line associated with the sea level diagram will be discussed later.

The yearly values of sea level in Figure 48 indicate quite clearly a more or less regular rise from the beginning of the series. However, the high and low waters appear to give contradictory indications. The high waters show a gradual decrease in height to 1930 and then a rapid increase, while the low waters show a rapid increase to 1933 and then a gradual decrease.

Taken at face value, the diagrams of Figure 48 might be interpreted as follows: the sea level data indicate a more or less progressive rise of about a quarter of a foot during the period 1922 to 1943; the high waters indicate a fall from 1922 to 1930 of about a quarter of a foot, after which there is a rise of more than half a foot; the low waters indicate a rise of nearly half a foot from 1922 to 1933 and then a slow fall amounting to less than a quarter of a foot.

The seemingly contradictory evidence shown by the high waters and low waters arises from the fact that in the use of the yearly values of these data no account is taken of the periodic variation in the rise

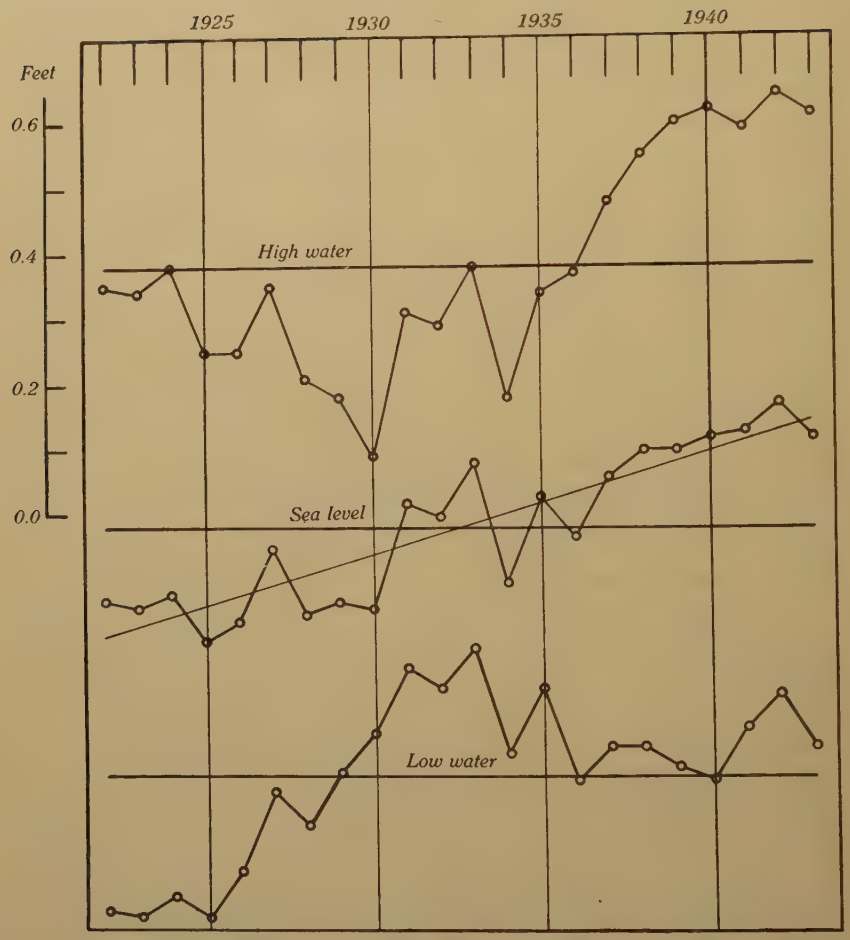

Figure 48. Yearly values of high water, sea level and low water at Boston.

and fall of the tide dependent on the variation in the longitude of the moon's node, which has a period of 18.6 years. The yearly heights of high and low water can be corrected easily for this variation (2). When this is done the yearly values of high and low water are as shown in Figure 49. The horizontal line associated with each of the diagrams represents the average value of the datum from the 22 corrected yearly values. Figure 49 shows that, after correction for the 18.6 year variation, the high and low waters both indicate approximately the same increase through the years as do the sea level heights in Figure 48.

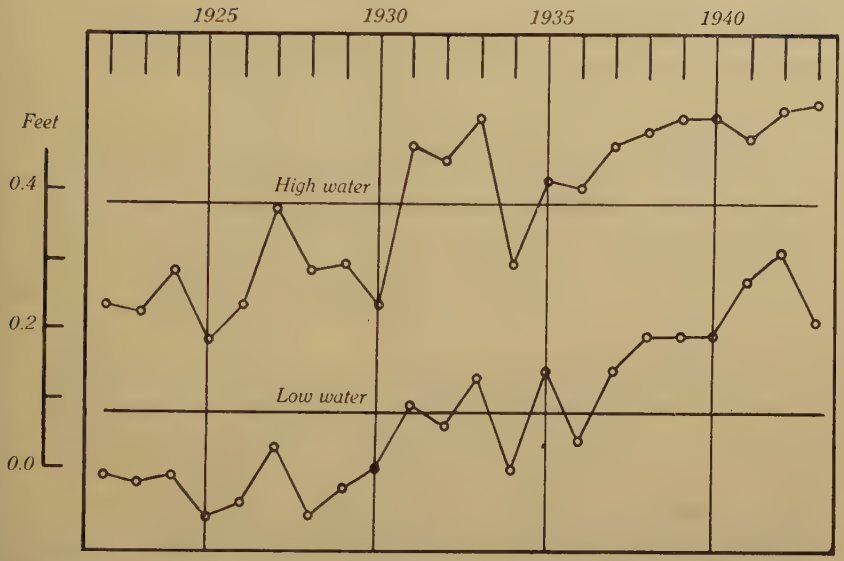

Figure 49. Yearly values of high water and low water at Boston, corrected for the longitude of the moon's node.

In the use of high or low waters in the study of coastal stability it is also necessary to investigate whether any change in the range of the tide occurred at the place of observation during the years in question. Such a change may occur from changes in local hydrographic features due to natural or man-made causes. Taking it all in all, the sea level data are much more satisfactory for the purpose. The high water and low water data have been included in the present paper to direct attention to the necessary procedures in their use, and to show that they corroborate the evidence from the sea level data.

The relatively large variations in the height of sea level from year to year shown in Figure 48 must be ascribed primarily to the effects of wind and weather. Heavy winds may raise or lower the level of the water in Boston Harbor by several feet. For use in the study of coastal stability it would be highly desirable to correct the yearly values of sea level to normal weather conditions. But at the present time this appears to be, practically, an unattainable ideal. However, we may determine the general trend of sea level for the 22 years of observation by fitting a straight line to the yearly values of sea level by the method of least squares. This is shown graphically in Figure 48 by the light sloping line associated with the sea level diagram, the calculation giving a rise of 0.0159 of a foot per year, or one-third of a foot for the 21-year interval from 1922 to 1943.

There is nothing in the data that permits extrapolation either beyond 1943 or before 1922. But light on the probable change in sea level at Boston prior to 1922 may be derived from a comparison of the Boston data with the sea level data at Baltimore, Maryland, for which place there are available tide observations since July 1902.

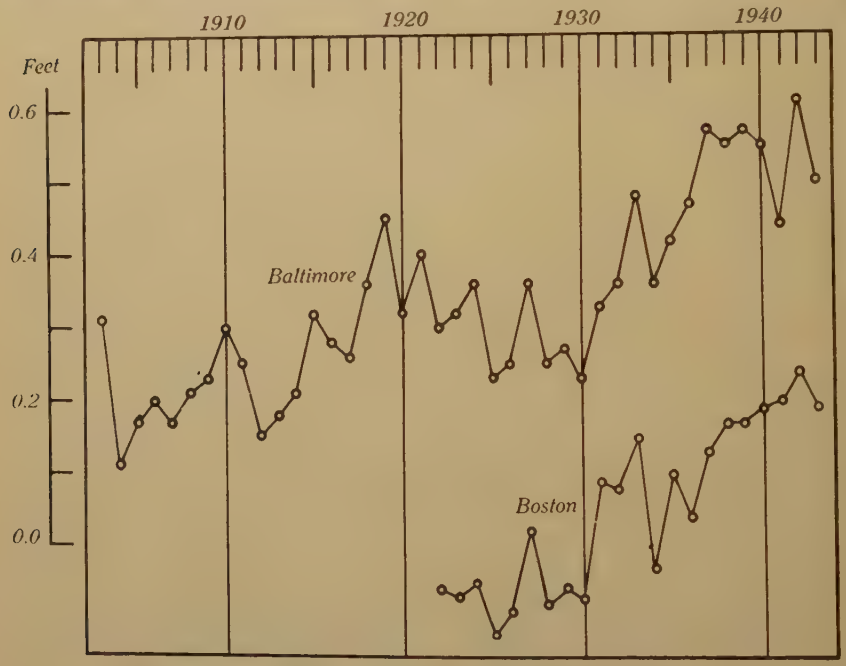

Figure 50. Yearly values of sea level at Baltimore and Boston.

In Figure 50 are shown the yearly sea levels at Baltimore and Boston for the years of observation at these places through 1943. By comparing the changes in sea level from year to year at the two places for the 22 years of simultaneous observations it will be seen that these changes are not exactly the same, although there is a rough parallelism. If we compute the rise in sea level for the period 1922–1943 at Baltimore by the method of least squares we derive a value of 0.0157 of a foot per year, as against the value of 0.0159 at Boston. Therefore, it appears reasonable to assume that the change in sea level, in recent years, has been practically the same at the two places.

For the 40-year interval from 1903 to 1943 a least-squares calculation gives for Baltimore a rise in sea level at the rate of 0.0086 of a foot per year. Hence, on the assumption that sea level at Boston

during the years 1903 to 1922 behaved in much the same way as at Baltimore, it must be concluded that the rise in the past 22 years has been at a more rapid rate than previously.

The change in sea level from year to year, as shown by the yearly values, is obviously very largely a reflection of the disturbing effects of wind and weather. The straight line fitted to the yearly values by the method of least squares completely ignores changes in the rate of rise of sea level. A curve giving an approximation to the probable change in sea level from year to year, freed from the larger effects of wind and weather, may be derived by smoothing the yearly values; but a much better approximation can be secured by smoothing the monthly values of sea level.

In a study of the sea level data at Baltimore the writer attempted to derive a curve for the most probable change of sea level from year to year by the use of a step-by-step smoothing of the monthly values of sea level. (3) This curve, revised slightly and brought up to 1943, is shown in Figure 51. For the purpose in view only five months, June to October, were used, since the sea level during these months is generally less disturbed by wind and weather than during the other months of the year.

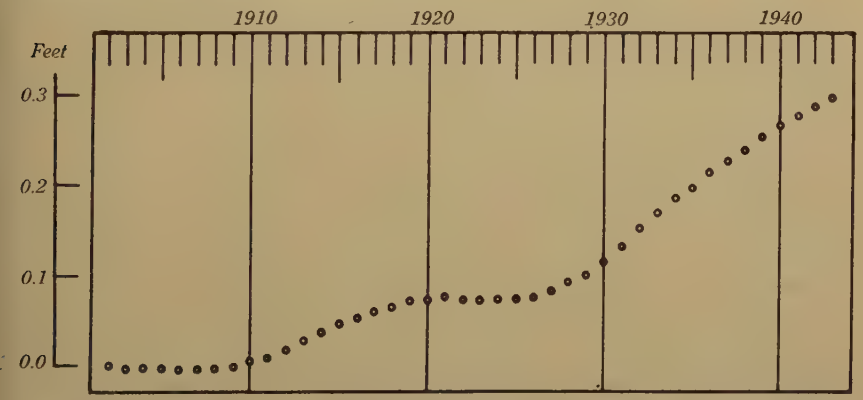

Figure 51. Yearly change in sea level at Baltimore, derived from smoothed monthly values.

In smoothing the monthly values of sea level, personal judgment enters as a factor, and therefore the method is not a rigorous one. Different investigators may derive curves differing somewhat in detail. But in its larger features this curve may be considered as a much better approximation to the real change in sea level from year to year than is given by the yearly values. On the assumption that at Boston

the change has been much the same as at Baltimore, Figure 51 may be considered as giving approximately the change in sea level at Boston for the period 1902–1943.

The least-squares calculation of the yearly sea level at Baltimore from 1922 to 1943 gave a yearly rise of 0.0157 of a foot, or a net rise for the 21-year period of 0.33 of a foot. From the curve in Figure 51 the net rise for the same period is 0.23 of a foot. The discrepancy between the two values is relatively large and emphasizes the caution necessary in the use of naked sea level values from observations covering periods of even 20 years duration.

A glance at the diagram of yearly sea level for Baltimore in Figure 50 shows that for the period 1922 to 1930 sea level as a whole was relatively low as compared with the preceding 5 years. Therefore the least-squares calculation for the period 1922 to 1943 gives a greater rate of rise than if the preceding years had been included. Hence, we may conclude that the value of 0.23 of a foot for the rise in sea level from 1922 to 1943, derived from the smoothed values in Figure 51, is a closer approximation to the real rise than is the value of 0.33 given by the least-squares calculation.

Now the relatively low sea level for the period 1922 to 1930, as compared with the immediately preceding years, is featured also by tide observations at Portland, Me., New York, N. Y., and Atlantic City, N. J. Therefore it is safe to assume that this was also the case at Boston, and that the yearly rate of rise of 0.0159 of a foot, derived from the least-squares calculation, is greater than the true rate if we take into account the immediately preceding years.

Considering Figure 51 as giving an approximation to the true change in sea level at Boston from 1902 to 1943, it is reasonable to conclude that sea level at Boston has been rising for the past 40 years, but at varying rates. Roughly we may divide the period into two intervals: 1902 to 1926 when sea level was rising at the rate of about 0.003 of a foot per year, and 1926 to 1943 when the rise was at the rate of about 0.013 of a foot per year. For the 41-year period the net rise was about 0.3 of a foot.

The sea level data at Boston do not distinguish whether this rise of sea level has been due to a subsidence of the coast, a general rise in sea level the world over, or a combination of the two. There can be no question that it is due partly at least to a world-wide rise of sea level. (1) As soon as careful sea level studies for various parts of the world become available it should be possible to determine how much is due to a rise in sea level and how much to local or regional subsidence of the coast.

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THE PHOTOMETRIC DETERMINATION OF NITRATE IN SEA WATER WITH A STRYCHNIDINE REAGENT

By

B. M. G. ZWICKER AND R. J. ROBINSON

Chemical and Oceanographic Laboratories
University of Washington, Seattle, Washington

INTRODUCTION

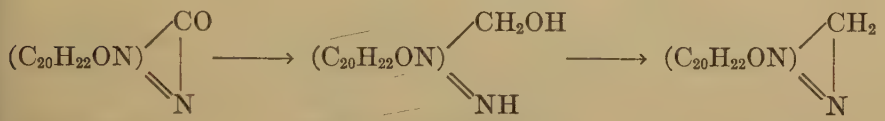
The routine determination of small concentrations of nitrate in water containing considerable chloride ion is limited to a few special procedures. Indirect reductive methods are usually avoided since natural waters contain other nitrogeneous substances. Direct estimations have been restricted to a few colorimetric procedures.

Of the several types of organic substances which give colored aqueous solutions with dilute oxidants, only one, the substituted aryl amines, has been found to have sufficient sensitivity to nitrate to be of use in the presence of significant chloride concentrations. Two substances of this class, diphenylamine (or diphenylbenzidine) and reduced strychnine, have been widely used in oceanographic work. Wattenberg (17) reported little difference between the sensitivity of Harvey's (7, 8) reduced strychnine method and Atkin's (2) diphenyl benzidine procedure. The reduced strychnine reagent, as prepared by customary methods, must be aged before being used, and therefore it is difficult to prepare reduced strychnine of reproducible sensitivity; furthermore the red oxidation color is not very stable.

The present investigation was undertaken in order to improve the reagent so that the method would be more useful in oceanographic work. The chemical nature of the active ingredients in reduced strychnine were studied, and the essential variables in the determination of nitrate in dilute solutions were investigated. The reduced strychnine reagent is shown to contain a mixture of reduction products. Of these, strychnidine is selected as having the greatest analytical possibilities. When using a strychnidine reagent the determination of nitrate is studied photometrically. In the oxidation of strychnidine, bistrychnidyl is probably first formed. The characteristics of bistrychnidyl as a reagent for nitrate are also presented.

EXPERIMENTAL

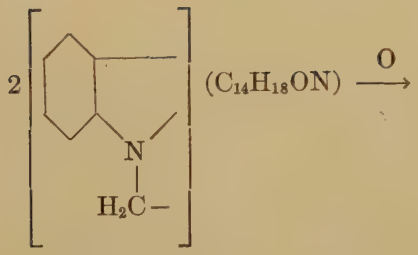
The reduced strychnine reagent. Tafel (13, 14) first reported the reduction of strychnine to a mixture of oxidant-sensitive alkaloids, and Denigès (5) discussed the preparation of such a reagent for the detection of nitrites and nitrates. Harvey prepared his reagent by the zinc amalgam reduction of strychnine. While a direct analysis has not been reported, Denigès, and more recently Giral and Ibanez (6), claimed the products to be a mixture of strychnidine and tetrahydrostrychnine. In the electrolytic reduction products of strychnine, Tafel (15) had found these compounds to be present in large quantities, as well as small amounts of other reduction products of a related nature. The relation of strychnidine and tetrahydrostrychnine is shown by the equation:



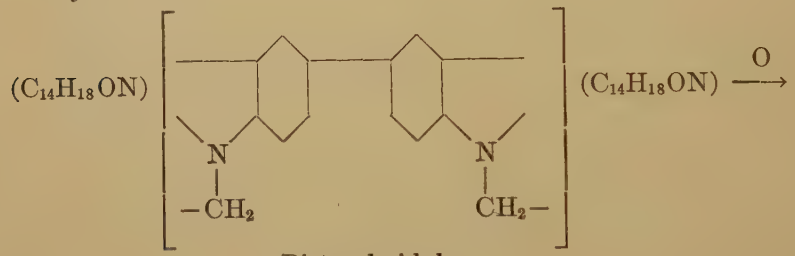
Strychnine Tetrahydrostrychnine Strychnidine

A reduced strychnine reagent was prepared according to the directions of Harvey (9) and immediately analyzed by a recrystallization procedure developed by the authors (21). Eighty-six per cent of the total alkaloid originally present was recovered. This mixture consisted of: 58% strychnidine, 18% tetrahydrostrychnine, 12% unconverted strychnine and 12% brown alcohol-soluble "dihydro-derivatives."

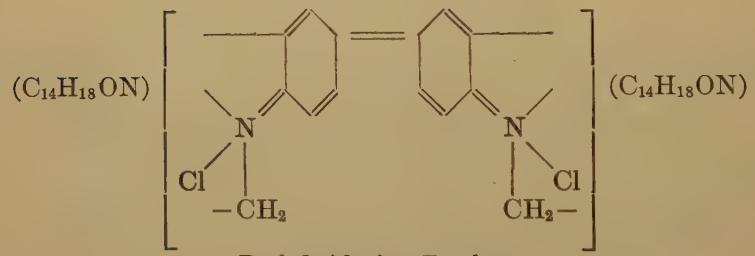
Achmatowicz and Robinson (1) have discussed the many types of reduced strychnine compounds and their color reactions with slightly acidic ferric alum solutions. The probable structure of these red oxidation products was established by Wieland and his co-workers (18). In their work on the structure of the strychnos alkaloids, colorless bis-compounds were prepared from both strychnidine and tetrahydrostrychnine which were "doubly sensitive" to oxidation. This reaction was explained by direct analogy to the quinoline-quinoid reaction of other substituted aryl amines such as diphenylamine or indole. In the case of strychnidine this reaction would be:



Strychnidine



Bistrychnidyl



Red Oxidation Product

Harvey (7) found that his reagent increased in sensitivity on aging six weeks. The authors observed, spectrophotometrically, that the red oxidation product from a sensitive, aged reagent gave a higher ratio of k_{5300}/k_{5000}* than that from the less sensitive, freshly prepared reagent whose analysis has been reported above. Using pure strychnidine and tetrahydrostrychnine prepared by the authors (21) from the electrolytic reduction of strychnine, the curves shown in Figure 52 were obtained from absorption measurements with a Bausch and Lomb spectrophotometer. The oxidation product of strychnidine in strong acid with either chromate or nitrate displayed a sharp maximum

* k_{5300} represents the specific extinction coefficient at the 5300 Å average spectrum cut with the S-53 filter of the Zeiss-Pulfrich photometer.

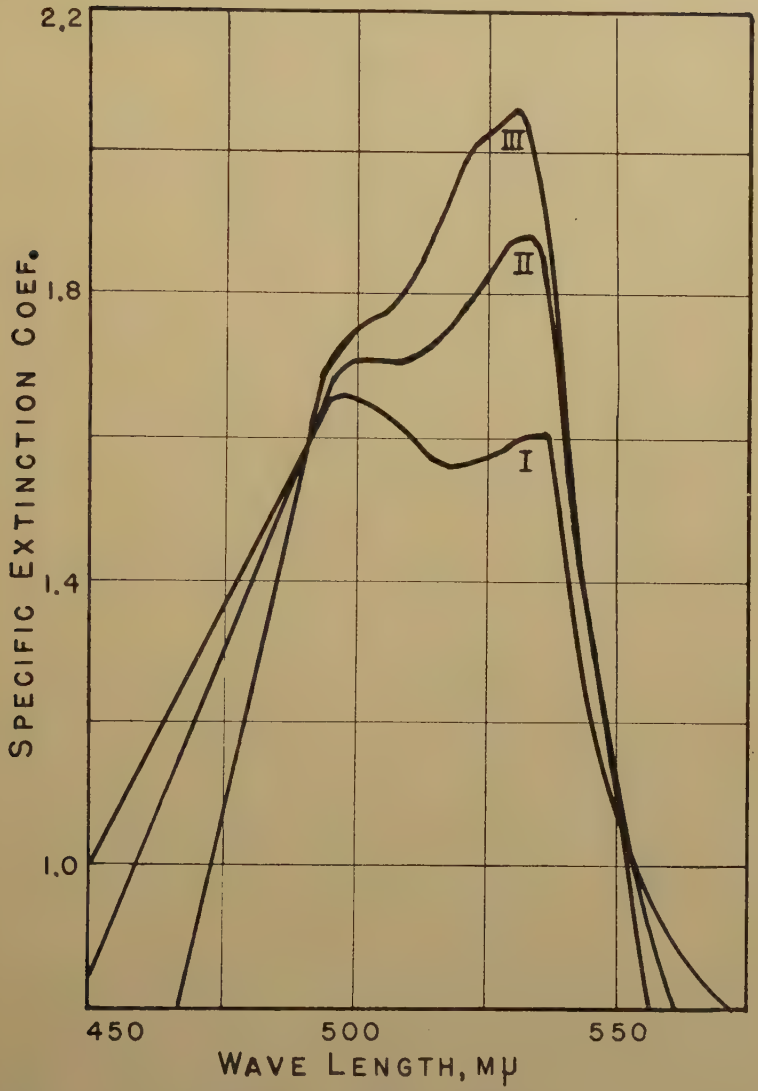


Figure 52. Absorption Spectra for Oxidized Strychnidine and Tetrahydrostrychnine· (I) Tetrahydrostrychnine, cold chromate oxidation; (II) Tetrahydrostrychnine, normal nitrate; (III) Strychnidine, normal nitrate.

at 5315 Å. When tetrahydrostrychnine was carefully oxidized with chromate in strong acid, two distinct maxima were observed, the one at 4980 Å being slightly higher than the second at about 5300 Å. By the usual oxidation with hot nitrate, variable curves were observed, lying between that of the cold reaction product and that of strychnidine. The maximum at 5300 Å was 10 to 30% greater than that at 4980 Å. Tafel (15) found that tetrahydrostrychnine could be converted to strychnidine by boiling with mineral acids, or better by treating with phosphorus oxychloride. The above evidence points to this conversion in the aging of Harvey's reduced strychnine reagent. Since even the most sensitive sample of Harvey's reagent that was available still displayed a prominent maximum at 4980 Å, it must be concluded that a complete conversion to strychnidine on standing was not obtained, and that probably other reduced strychnine derivatives were present also.

The strychnidine reagent. The selection of strychnidine as an improved reagent for nitrate was based on the above results. Not only does strychnidine produce a dye of more intense absorption maximum, (see Figure 52), but the colored oxidation product is largely limited to a single species. Also, the 5300 Å absorption band lies close to the maximum sensitivity of human vision at low light intensities.

Preparation of Strychnidine. Strychnidine was prepared from strychnine by electrolytic reduction on a mercury cathode (21). About 10 g of strychnine sulfate, U.S.P. XI were dissolved in 55 ml of 64% sulfuric acid (sp gr 1.54). The cooled solution was placed in the cathode compartment of a reduction cell containing 17.8 cm^2 of mercury cathode. The porous anode compartment was filled with 60% by weight sulfuric acid and a strip platinum anode of 15 cm^2 surface inserted. A temperature of 25° C was maintained during reduction at 0.75 ampere, 5 terminal volts and 3 cathode volts. The reduction was continued for 35 hours to increase the yield of strychnidine over tetrahydrostrychnine.

The catholyte was diluted with 83 ml of 0.01% sulfuric acid to give 28.5% sulfuric acid. On cooling to room temperature, or colder, most of the unreduced strychnine was precipitated as the acid sulfate (20). This was filtered and washed with a small amount of 28.5% sulfuric acid. The excess acid was slowly neutralized with concentrated ammonia, 100 to 200 g of ice being added to cool the solution. The amorphous base, largely strychnidine and tetrahydrostrychnine, was digested at 40° to 50° C to remove excess ammonia and to dissolve the tetrahydrostrychnine. The strychnidine residue was filtered and purified further with two extractions of 100 ml portions of warm water.

To increase the yield the aqueous extracts were concentrated, the crude tetrahydrostrychnine extracted by chloroform and then mixed with ten times its weight of phosphorous oxychloride under an atmosphere of carbon dioxide. After refluxing 45 minutes at 60 to 70° C under reduced pressure, the excess reagent was removed by vacuum distillation. The strychnidine was obtained from the diluted brown syrup by precipitation with ammonia, or as the hydriodide.

The crude strychnidine was recrystallized from 100 ml of 95% ethyl alcohol and finally recrystallized slowly two or three times from thiophene-free benzene to give pure strychnidine, m. p. 256.6° C in vacuo.

Photometric determination of nitrate. A reagent of one millimole of strychnidine (0.320 g per liter) is recommended for nitrate concentrations up to 50 μg at.[1] NO_3-N/liter. The strychnidine reagent was prepared from the colorless prisms of strychnidine. The alkaloid was weighed directly into a volumetric flask and oxidant-free sulfuric acid (sp gr 1.84) was added immediately. The solution was kept in an aged brown glass bottle. Sulfuric acid, manufactured by the contact process, is the most convenient to use. If acid of sufficient purity is not available, nitrates may be removed with ammonium sulfate according to Cooper (4) or with hydrogen sulfide according to Atkins (3).

A stock solution of 750 μg at. NO_3-N/liter was prepared from recrystallized C.P. sodium nitrate that had been dried at 110° C. The stock solution was made to contain 25 parts of chloride-ion per liter with 20 ml of a saturated solution of C.P. mercuric chloride solution per liter as a preservative and twice recrystallized sodium chloride. The standard nitrate solutions were prepared from the stock solution by appropriate dilution with sodium chloride solutions containing 25‰ Cl, i. e. grams Cl per 1000 grams of solution.

Equal volumes of reagent (in concentrated sulfuric acid) and nitrate solutions were carefully mixed in well aged pyrex tubes by four careful but rapid transfers from one to the other. The hot mixtures were immediately placed in the dark for three to five hours. Measurements were made with the Zeiss-Pulfrich photometer; the 0.1–15 mm. variable depth cell was convenient for the present investigations, and a 10 mm cell containing distilled water was used for the reference standard. A blank determination for the variable cell was made with 60% by weight sulfuric acid. A preliminary check comparison was always made on the S-50 filter before the measurement with the S-53 filter; repetition of the readings was always necessary

[1] A microgram atom, μg at., is one millionth of a gram atomic weight.

unless the operator's eyes had been "conditioned" by a series of readings. Operation in a darkened room was found effective in increasing efficiency and eye sensitivity.

Concentration of the reagent. To 3.0 ml portions of standard nitrate solution of 75 μg at. per liter was added 3.0 ml of 38% sulfuric acid containing increasing amounts of strychnidine reagent. The results are shown in Table I and Figure 53. At lower concentrations of the reagent Beer's law was followed closely. Finally maximum color was obtained and further increases in the ratio of strychnidine to nitrate resulted in gradual decrease of color. Fading with time was more rapid at the higher reagent concentrations.

The increase in specific extinction coefficient per mole of strychnidine concentration in the mixture (i. e., molar extinction coefficient) is equal to the slope of the linear part of the curve, which was found to be 20,500. The intercept of this linear portion with that showing maximum color formation indicated that at least fourteen moles of the strychnidine are required for reaction with one of nitrate.

Table II and Figure 54 show standardization data for reagents of one and six millimoles of strychnidine per liter over a range of 2 to 75 μg at. NO_3-N per liter. Although divergencies from a linear curve are less than 5%, at lower concentrations there is a tendency for lower absorption values with the higher reagent concentrations. The standardization curve for the best Harvey's reduced strychnine reagent is included to show its linearity at a lower sensitivity, as well as the curve for the bistrychnidyl reagent at nearly double sensitivity.

A reagent of one millimole of strychnidine per liter is recommended for nitrate concentrations between 5 and 50 μg at. per liter.

Time required for color development. The effect of time on the color development is shown in Figure 55 for three typical nitrate concentrations. The time required for maximum color formation increased with increasing nitrate concentration. The greater instability of the color with high nitrate concentration was typical. Harvey (8), Sund (12) and others have found that Beer's law failed to hold above 25 to 30 μg at. NO_3-N per liter with Harvey's reagent, but they measured the color 12 to 24 hours after mixing. With pure strychnidine, Beer's law failed above 50 μg at. NO_3-N per liter with a reagent concentration of 2 millimoles per liter in 24 hours.

Three to five hours were the most satisfactory for color measurements over a wide range of concentrations. Harvey's reagent required about 20% longer for the high nitrate concentrations than was noted with strychnidine.

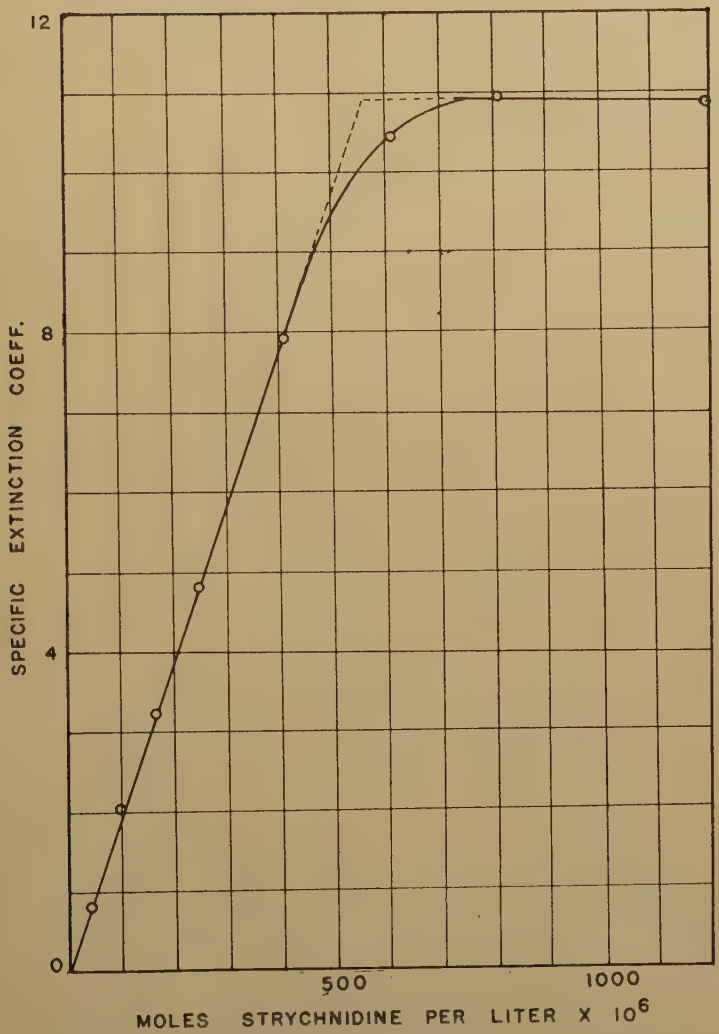


Figure 53. Strychnidine Requirements for 75 μg at./l. NO_3-N.

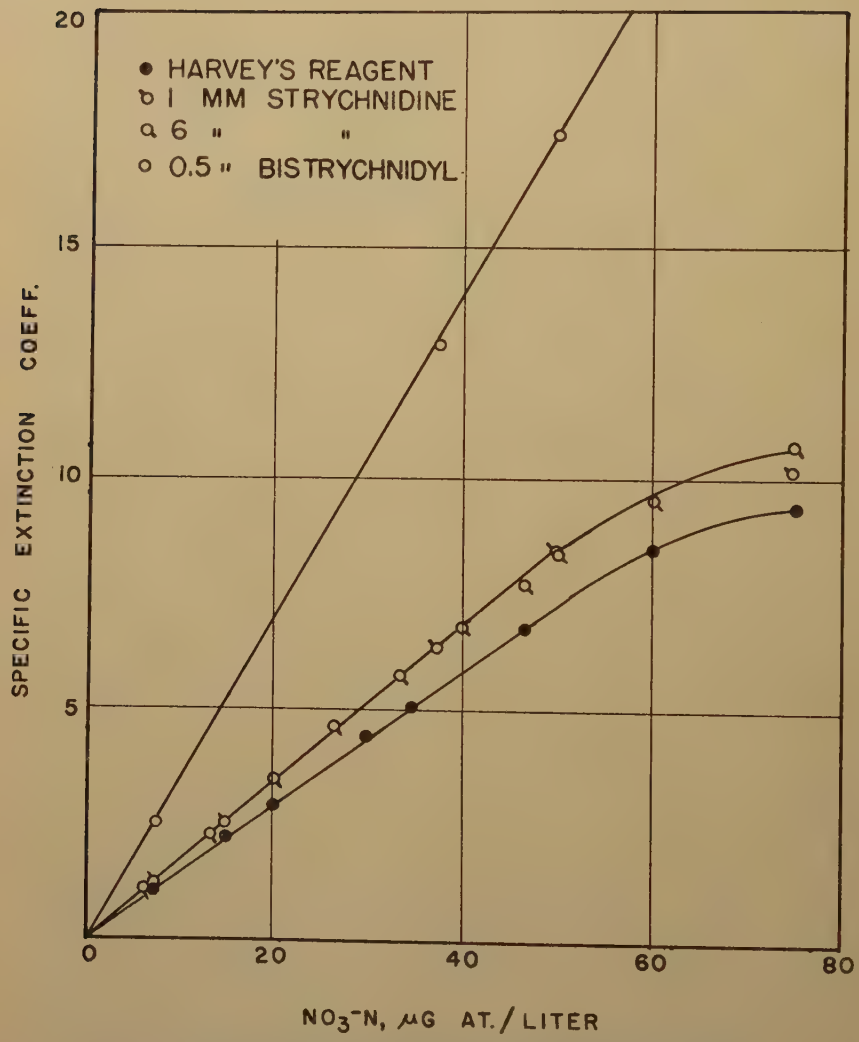


Figure 54. Standardization Curves at Four Hours Color Development.

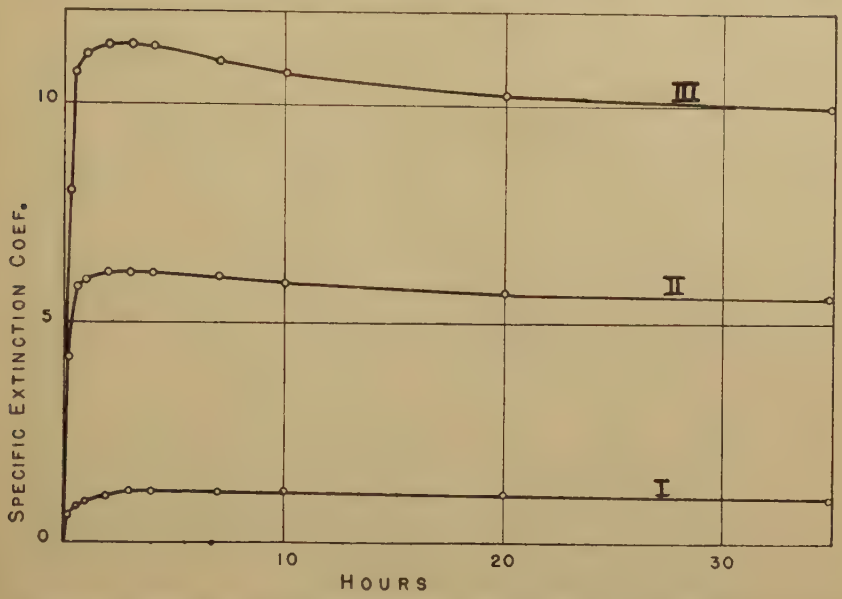


Figure 55. Development and Fading of Color with 1 millimolar Strychnidine Reagent. (I) 7.5 μg at./l. NO_3-N; (II) 37.5 μg at./l. NO_3-N; (III) 75 μg at./l. NO_3-N.

Characteristics of the colored oxidation product of strychnidine. Spectrophotometric absorption data were obtained for the pure strychnidine, m p 256.5° C in vacuo, with a Bausch and Lomb spectrophotometer, oxidized in dilute and concentrated acid by dilute chromic acid; the curves are shown in Figure 56. The maximum at 5315 Å was more intense in strong acid, while the maximum at 4980 Å was more pronounced in weak acid.[1] Further evidence of two oxidation products is shown in Figure 57 in which specific extinction coefficient for the 5300 Å is plotted against time for several concentrations of acid. These data were obtained with the Zeiss-Pulfrich photometer. All the solutions were mixtures of equal volumes of 0.001 molar strychnidine in sulfuric acid and 0.00005 molar ferric alum.

Part of the above data were predicted qualitatively by Achmatowicz and Robinson (1), who claimed that the color produced with ferric alum and strychnidine decreased with an increase of acidity from 0.01 to 0.1 N. However, the change in color and the increase in intensity in strongly acid solutions has not been reported.

[1] The colors were visually quite different. At low acid concentrations an initial salmon red faded to a light yellow, while in concentrated acid a bright cherry red was produced.

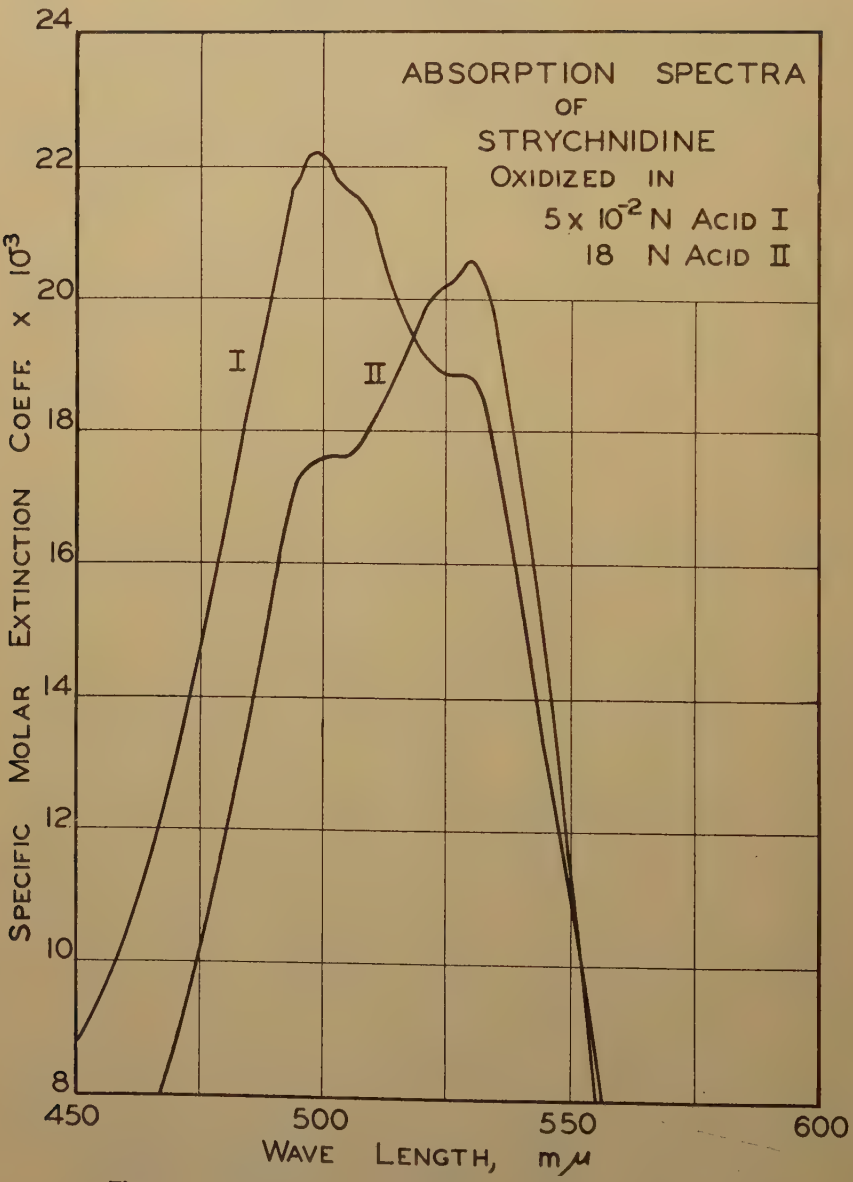


Figure 56. Effect of Acidity on Absorption Spectra of Strychnidine.

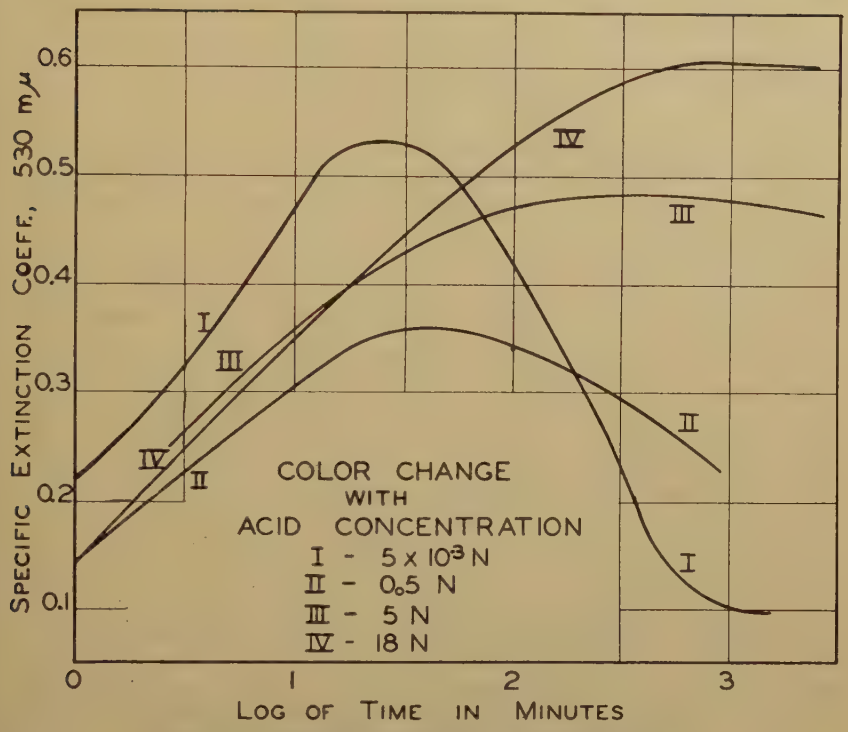


Figure 57. Effect of Acidity on Color Change with Time.

Contrary to the conclusions of Tafel (14) and Achmatowicz and Robinson (1), the color produced in dilute acid solutions was found to be quite heat labile. In dilute solutions it faded rapidly in the dark, even at room temperature, and in more concentrated solutions, a pure yellow to buff solution was obtained in one-half hour at 100° C. The solutions used by the above authors were considerably more concentrated than those in which the fading was so strikingly displayed, but photometric analysis of similarly concentrated solutions showed that there was a decided decrease in color in an hour at 100° C, and the mixture became quite brownish in hue as the heating was continued.

In strong acid solution the more brilliant crimson color was considerably more stable to heat and light, although three to four hours at 100° C. was sufficient to convert this to a brown or buff. Further evidence of the more stable nature of this oxidation product formed in concentrated acid solution was obtained by the addition of aliquots of sulfurous acid solution to equivalent quantities of dye at several

acid concentrations. In 0.01 N acid, the red solution was immediately decolorized, but in 15 N sulfuric acid the dye was completely reduced only after several days of repeated treatments.

The addition of concentrated sulfuric acid to a dilute acid solution of oxidized strychnidine, either before or after fading had occurred, resulted in the rapid formation of the stable red dye. Dilution of the strong acid solution of the dye caused it to fade rapidly, especially in the presence of light, but the absorption spectrum was not affected.

The spectrophotometric absorption curves for strychnidine and tetrahydrostrychnine oxidized with chromate, vanadate, iodate, bromate, chlorate, permanganate, manganate, peroxide, ferricyanide, ferric or ceric ions, proved to be identical with those from nitrate and nitrite. This evidence supports Wieland et al. (18) in their explanation of the structure of the dye. According to this mechanism, the easy conversion of the salmon red dye formed in dilute acid to the crimson dye in concentrated acid might be associated with the basic nature of the indole nitrogen. The previous reactions given above show the oxidation of strychnidine to the meriquinoid dye.

The effect of chlorides on color formation. Harvey (7) reported that a well aged reagent was insensitive to chloride concentration above 6°/₀₀, but Riddell (10) prescribed the addition of 0.5 ml of concentrated hydrochloric acid to each 7 ml of sea water sample to obtain maximum sensitivity. Preliminary work of the authors (19) on this subject, using Harvey's reagent, indicated that at least 25°/₀₀ Cl was necessary for concentrations of nitrate greater than 20 μg at. NO_3-N per liter to obtain maximum sensitivity.

Using a millimolar solution of strychnidine, the results shown in Figure 58 were obtained. The low nitrate concentration required more chloride for the maximum value than the higher concentrations, but at the same time the higher concentrations were affected more critically by the excess chloride concentrations. For the region of nitrate concentration around 7.5 μg at. per liter, a molar Cl/NO_3 ratio of at least 5,000 (Cl = 1.5°/₀₀) was necessary, but it could be as large as 19,000 (Cl = 6°/₀₀) without affecting color formation. The range that was usable for 37.5 μg at. NO_3-N per liter was found to be between Cl/NO_3 = 1,900 (Cl = 2.5°/₀₀) and Cl/NO_3 = 18,800 (Cl = 25°/₀₀). The usable range for 75 μg at. NO_3-N per liter was found to be between Cl/NO_3 = 13.3 (Cl = less than 1) and Cl/NO_3 = 5,600 (Cl = 15°/₀₀). This accounts for the apparent failure of the reagent in determinations using the usual nitrate procedure. The increase of the concentration of the strychnidine to 5 millimolar failed to change the standardization curve up to 75 μg at. NO_3-N per liter, for chlorinities between 5

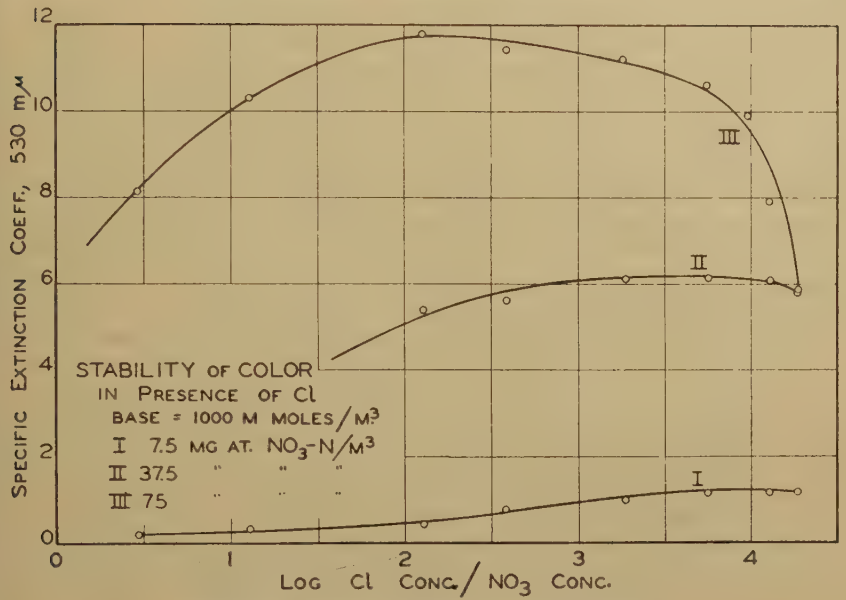


Figure 58. Effect of Chlorinity on Color Stability.

and 25°/₀₀. Since the normal nitrate concentrations in sea water vary between 5 and 40 μg at. per liter with chlorinities of 16 to 20°/₀₀, there appears to be no need for added chlorides when a pure strychnidine reagent is used, even in quite brackish waters. For fresh water, the addition of some chloride is essential.

Stoichiometry of the reaction. The data presented in Table I and Figure 53 indicate an abnormal reaction with nitrate in strong acid solution. If Wieland's reaction is correct, the oxidation of the meriquinoid compound would require two oxidizing equivalents per mole of strychnidine. The indicated reaction of about sixteen moles of strychnidine with each nitrate is not consistent with the known oxidizing power of the nitrate alone, even assuming reduction to ammonia and complete oxidation to the red dye.

The action of other oxidants on strychnidine in various acid concentrations was studied with inconclusive results. The concentration of both the strychnidine and the oxidant affected the color formation. The presence of chlorides reduced the color formation in many instances and the acid concentration also affected the results. In all cases the spectrophotometric absorption curves of the strong acid oxidation products were identical with those obtained by nitrate.

The maximum color formation per oxidizing equivalent in strong acid was only one-sixteenth the amount of color obtained per gram mole of nitrate in the case of vanadate, permanganate, ferricyanide, ferric or chromate. Nitrite was an exception, since it gave from one-half to three-quarters as much color as an equimolar quantity of nitrate.

Stoll (11) has discussed the relative effects of ferric and manganic ions in relation to that of nitrates on Harvey's reagent, and a recalculation of his data showed that each nitrate-nitrogen was equivalent to about 4.5 ferric ions and 2.6 manganic ions. Harvey (8) noted that nitrites produced variable color formation, from 50 to 80% of that formed by its equivalent of nitrate.

Some sources of error. When Harvey's reagent was used with sea water it was necessary to centrifuge each mixed sample in order to remove a white precipitate before comparison was made photometrically. This precipitate was not observed when the strychnidine reagent was used.

Surface waters often contain considerable suspended organic matter. In an investigation of plankton cultures, check analyses were not obtained unless the water was filtered through a fritted glass crucible of fine porosity (plate pores 5 to 10 microns).

The presence of dissolved organic matter and inorganic reducing agents, especially in brackish and polluted waters, probably will result in erroneously low nitrate values. The addition of some easily oxidized compounds such as oxalic acid, glucose or sulfites to standard nitrate samples lowered the color formation materially. Sund (12) in discussing the problem, advised the addition of two different quantities of barium nitrate to brackish water, whereby the effect of reducing substances could be approximately determined by calculation.

Strychnidine reacts in acid solution with nearly all common oxidants to form the red dye which is spectrophotometrically identical to that formed with nitrate. Harvey (7, 8), Thompson and Johnson (16), Cooper (4) and others have considered the presence of oxidants other than nitrates in sea water. They have agreed that nitrite-nitrogen is the only one that is usually present in sufficient quantities to be effective. Since its concentration is normally less than 5% of that of nitrate-nitrogen, its presence is not a serious matter.

Sensitivity and accuracy. The ultimate sensitivity of the strychnidine reagent has been determined to be about 0.2 μg at. NO_3-N per liter with standard nitrate solutions. However, this sensitivity is not practical, since the blank determination is usually materially greater than this value, and large volumes of solutions would be required for

the photometric analysis. The practical limit of sensitivity may be considered to be 0.5 to 1 μg at. NO_3-N per liter with the Zeiss-Pulfrich photometer.

As little as 1 ml of sample has been used with success; if samples of smaller volume are desired, dilution with 60% sulfuric acid is recommended. Blank determinations are required, of course.

Using the procedure described above, an experienced operator has been able to duplicate his results consistently within the accuracy of the Zeiss-Pulfrich photometer. Under the optimum conditions of measurement an accuracy of ± 1.5% may be expected. Table III summarizes the authors' recommendations for analysis of various ranges of nitrate concentration in order to utilize the optimum operating conditions of the reagent and instrument.

Bistrychnidyl reagent. From a study of the reactions summarized in the first part of the paper it is evident that the disadvantages of the strychnidine reagent might be largely eliminated by the use of bistrychnidyl as the active reagent. Attempts to prepare a pure sample of bistrychnidyl for such a purpose were not successful. Its crystalline form was poor and the samples were invariably contaminated with brownish-red oxidation products.

A reagent was prepared by the following procedure. This product was nearly colorless and exhibited the desired properties of sensitivity (see Figure 54).

Dissolved in a small volume of 0.5 N hydrochloric acid at 0 C° in an atmosphere of carbon dioxide was 0.160 g of pure strychnidine. To this were added slowly over a period of 10 minutes 1.1 milliequivalents of chromic acid (11.0 ml of a 0.10 N H_2CrO_4 solution). Dilute sodium bisulfite solution was then added dropwise at 0° C until a light green color was obtained. Sulfuric acid (sp gr 1.84) was added slowly, an ice bath maintaining the temperature below 5° C, until the solution had a volume of 500 ml. Very dilute chromic acid was then added until the complete removal of the excess sulfite was indicated by the appearance of a very faint pink tinge. The reagent had to be stored in an aged brown glass bottle under an atmosphere of carbon dioxide, otherwise its blank slowly increased.

The colors obtained with bistrychnidyl formed more rapidly than with strychnidine; comparison could be made after an hour in the dark. The effect of chloride was as marked as in the case of strychnidine, but fading in the dark was slow with this reagent. An increase in color was noted with low nitrate concentrations upon standing in the laboratory for 24 hours, indicating a slow air oxidation. This extreme sensitivity should make possible a material reduction in the sulfuric acid concentration necessary for the determination.

SUMMARY

A reagent prepared from strychnidine has been examined for the photometric determination of NO_3-N in sea water. It was found to be more stable, reproducible and sensitive than Harvey's reduced strychnine reagent, which was shown to be a mixture of reduction products. The effects of reagent concentration, time, temperature, light, acidity and chlorinity on the color production have been studied. Recommendations have been made concerning the technique of the method for use with a Zeiss-Pulfrich photometer. The errors, sensitivity and accuracy of the procedure have been discussed. A reagent prepared from bistrychnidyl was found to be even more sensitive than the strychnidine reagent, but somewhat difficult to prepare and use.

TABLE I—Effect of Reagent Concentration

Strychnidine Concentration Molarity $\times 10^6$	*Strychnidine Nitrate*	*Specific Extinction Coefficient with S-53 Zeiss filter*
4010	95.0	9.87
3010	71.0	10.15
2005	47.5	10.71
1600	38.0	10.97
1200	28.4	10.85
800	19.0	10.95
600	14.3	10.46
400	7.60	7.91
240	5.65	4.81
160	3.80	3.22
99	2.35	2.04
40	0.95	0.81
7	0.18	0.04

TABLE II—Standardization of Reagents (25% Cl; After 4 Hours)

Nitrate	*Specific Extinction Coefficient, S-53*			
μg. at. per l.	*Harvey's*	*Strychnidine*		*Bistrychnidyl*
		1 millimolar	*6 millimolar*	*0.5 millimolar*
6.7	....	.....	1.11	.....
7.5	1.10	1.25	.....	2.55
13.3	....	.....	2.27	.....
15.0	2.25	2.55	.....	.....
20.0	2.90	.....	3.48	.....
26.7	....	.....	4.64	.....
30.0	4.39	.....	.....	.....
33.3	....	.....	5.75	.....
35.0	5.04	.....	.....	.....
37.5	....	6.35	.....	12.9
40.0	....	.....	6.75	.....
46.7	6.75	.....	7.70	.....
50.0	....	8.55	8.40	17.4
60.0	8.50	.....	9.55	.....
75.0	9.40	10.2	10.7	24.6
100.0	....	11.6	.....	.....

TABLE III—Recommended Procedure for the Photometer

NO_3 Range μg at. per l.	Strychnidine Millimoles per l.	Sample in ml.	Size of Test tube in mm.	Time of Standing in hours	Thickness of Cell stratum in mm.
0.2 to 4	0.10*	14	20 x 155	4–24	50
2.5 to 10	0.10*	3	13 x 100	2– 8	10
5 to 15	0.50	3	13 x 100	2– 5	5
12 to 40	1.0	3	13 x 100	2– 5	2
25 to 75**	5.0	3	13 x 100	2– 4	1

* Bistrychnidyl reagent is recommended for this range (see below).
** Samples above 50 μg at. NO_3/N per liter should be diluted for best results.

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